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TIRC

Grants

1954-60

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STANDARD

STANDARD B & P "NOISE" 

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November 4, 1955

Memorandum to

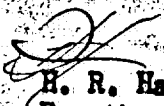
Dr. Robert C. Hockett
Associate Scientific Director
Tobacco Industry Research Committee
5320 Empire State Building
New York 1, New York

Below are our comments on Research Grant Application forwarded with your memorandum of October 28.

No. 115 - Dr. Clinton H. Thienes

Since the first part of this project does not include any experimental work - only a recalculation of original data which has already been published, republication of such material may be somewhat difficult unless incorporated into the second part of the project.

In part two assay of the adrenals at more than one time interval following nicotine injection might be recommended to provide information on the rate of recovery of the epinephrine and nor-epinephrine content of the gland. The effect of anesthesia on the hormone content of the adrenal medulla may also require evaluation.


H. R. Hammer
For the Sub-Committee of the
Industry Technical Committee

VH

cc: Dr. R. N. DuPuis ✓

I have taken the liberty of forwarding these comments to Dr. Hockett.

H.R.H.

1003537079

Application For Research Grant

no
Date: September 29, 1955

1. Name of Investigator:

Clinton H. Thienes, M.D., Ph.D.

2. Title:

Director

3. Institution

& Address:

Institute of Medical Research, Huntington Memorial Hospital,
734 Fairmount Avenue, Pasadena 2, California

4. Project or Subject:

Effect of Daily Nicotine Administration Upon Adrenal Glands,
and Upon Mortality of the Newborn.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

This is a two fold problem, for which much of the "raw data" have been obtained. Twenty-five pairs of rats were given twice daily injections of saline containing nicotine for a period of a year. Their litter mates (another 25 pair) were injected with saline alone, as controls. The number of young born were tabulated and a report of the effect of nicotine on fertility was made in the Journal of Pharmacology and Experimental Therapeutics, 87:1, July, 1946. A rough estimate indicated that there was a marked increase in mortality of babies born to nicotine treated mothers but the raw data on this point have never been adequately studied and evaluated.

Furthermore, data on the adrenal glands needs further evaluation. A rough estimate indicates no actual increase in size, but an increased ratio between size and adrenal weight and body surface. Earlier work, published in the Journal of Pharmacology, 46: 113, 1932, showed a ~~dx~~ decrease in body weight due to ~~dx~~ decrease in fat (hence of body surface) of nicotine injected rats, without a decrease in skeletal size. The results of our last experiments indicate therefore that the increase in adrenal weight/body surface ratio in nicotine injected rats does not indicate an actual increase in size of the adrenals, but only a decrease in the body fat. We need to recalculate our raw data to determine if this impression is true.

The second part of the problem deals with tolerance development to the effects of nicotine on the secretion or formation of the adrenomedullary hormone. Earlier, unpublished work, now realized to have been too inaccurate, indicated (1) that a

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nicotine injection decreases the epinephrine content of the adrenal gland and (2) that this effect is greatly reduced in animals chronically treated with nicotine. We would like to repeat this experiment, on rats, using modern methods of epinephrine (and nor-epinephrine) assay, both biological and chemical. The rats would be injected twice daily for six months with a nicotine-in-saline. They would then be ~~maxima~~ anesthetized with pentobarbital. The adrenals of one half of the test group and of the control group would be assayed for epinephrine and nor-epinephrine; the other half of each group would be injected with 0.5 mg. of nicotine per Kilogram body weight, and the adrenals removed 10 minutes later and assayed for the medullary hormones.

In view of the demonstrated tolerance of the central nervous system to nicotine (Jour. Pharmacol. and Exper. Therap. 48:317, 1933) it is important to know what other types of tolerance occur.

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6. Budget Plan:

Salaries + 5% Employee benefits	\$4200
Expendable Supplies	375
Permanent Equipment	250
Overhead (40% of Salaries)	1600
Other Travel & Publications	500
Total	\$6925

7. Anticipated Duration of Work:

one year

8. Facilities and Staff Available:

Animal quarters, laboratory space, kymographs, photo-electric colorimeters, Coleman Jr. Spectrophotometer, Beckman DU Spectrophotometer, constant temperature baths, etc. necessary for chemical and biological tests. Staff consists of director, head technician, secretary, animal man and varying numbers of professional and technical persons and graduate students depending on number and size of projects. A technician or two graduate students will be needed for the project on nicotine.

9. Additional Requirements:

None

10. Additional Information (Including relation of work to other projects and other sources of supply):

This project is not related to other projects of the Institute of Medical Research

s/ Clinton H. Thienes
Signature _____
Director of Project

s/ Alan R. Baldwin
Business Officer of the Institution

Recorder

Controller

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COPY

INSTITUTE OF MEDICAL RESEARCH
HUNTINGTON MEMORIAL HOSPITAL
734 Fairmount Avenue
Pasadena 2, California

September 29, 1955

Robert C. Hockett, Ph.D.
Tobacco Industry Research Committee
350 Fifth Avenue
New York 1, New York

Dear Dr. Hockett:

Herewith is application for a grant-in-aid for an investigation of chronic nicotinism. It may be of interest to your committee members to have information on the Institute of Medical Research of the Huntington Memorial Hospital, since it is but three years old.

The Institute is housed in a reinforced concrete building, two stories in height, and with a complete basement. There are two general laboratories, each large enough for six people, nine laboratory cubicles of different sizes, a well equipped animal surgery, four animal rooms, a cage cleaning room and a food room. The laboratories are well equipped with not only standard physiological and biochemical equipment, but many special items such as three spectrophotometers, one of which is equipped with an automatic recorder, a refrigerated centrifuge, etc. The total budget has averaged about \$100,000 per year since January 1953. Each research project is supported by a grant-in-aid or by a gift from an interested person. The director was for twenty-three years professor of pharmacology and toxicology at the University of Southern California and is now visiting professor.

Sincerely yours,

s/ Clinton H. Thienes

Clinton H. Thienes, M.D.
Director

CHT:amh

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TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East Forty Second Street

New York 17, N.Y.

Application For Research Grant

#203R1

cf. #89

activated 10/1/55

renewed 7/1/56

#168

activated 9/1/57

#203

activated 9/1/58

Date: May 11, 1959

1. Name of Investigator: Dr. Caroline Bedell Thomas
2. Title: Associate Professor of Medicine
3. Institution & Address: The Johns Hopkins School of Medicine
710 North Washington Street
Baltimore 5, Maryland
4. Project or Subject:
 - a. Studies of Genetic Differences between Smokers and Nonsmokers.
 - b. Studies of Psychological Differences between Smokers and Nonsmokers as Shown by Comparison of Figure Drawings.
5. Detailed Plan of Procedure:

The background for our future plans may be found in Attachments I, II and III. Attachment I gives the original plan for the two year study as presented in our application to the TIRC in 1958. Attachment II indicates briefly the progress made in each phase of the present studies this year. Attachment III summarizes progress made in 1958-1959 in our over-all program and lists the publications. In III, note particularly sections A4; C2, 3, 4 and 5; E2a and b, 3a, 4a, and 5 a and b. The paper listed in 3a has now been published, but reprints have not yet been received. Work is in progress on 5a and b; it is hoped that these papers will both be submitted for publication before September, 1959. Reprints for the papers listed under 2a and b are attached. The latter work was not primarily supported by the TIRC nor did Dr. Murphy (the part-time Fellow supported by the TIRC) have any part in the design or execution of the experiment or in the writing of the paper per se. His contribution was to make a statistical analysis of the data in Table III at a time when other statistical assistance was not available.

Plans for the coming year:

A. Studies of Genetic Differences between Smokers and Nonsmokers.

1. to continue the orderly collection of data from blood donors as has been done this year (see Attachment II).
2. to try out taking smoking histories by a self-administered questionnaire (see card at end of Attachment II) rather than by interview. If this proves satisfactory, the data on blood groups in smokers versus nonsmokers can be expanded to include many more donors than it is possible to interview. (Our interviewers are at the Blood Bank about 4 hours a day five days a week.)

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3. to make a preliminary analysis of the data thus far collected in the near future in order to obtain an estimate of the total number of subjects it will be desirable to include in the entire study.
4. when data collection has been completed, to make a statistical analysis of the results and write them up.

B. Studies of the Psychological Differences between Smokers and Nonsmokers as Shown by Comparison of Figure Drawings.

1. to complete the preliminary study with Dr. Greenberg described in Attachment IIB.
2. to make a statistical analysis of the smoker-nonsmoker comparisons for the variables coded on IBM cards (see Attachment IIB).
3. to incorporate the results of these two studies into one or two papers.

6. Budget Plan:

Salaries	5,500.00
Expendable Supplies	222.25
Permanent Equipment	
Overhead (15%)	1,500.00
Other	4,278.75
Total	\$11,500.00

7. Anticipated Duration of Work: one year.

8. Facilities and Staff Available: Dr. C. Lockard Conley and Dr. Julius R. Krevans, who are in charge of the blood bank have given us permission to make use of blood bank donors and blood group data in the manner indicated. There is ample work space available in conjunction with our major project. During the next two years, we shall have a full-time statistician working with us. A part-time psychologist is already on our staff.

9. Additional Requirements: none.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The aims of the project outlined above are in harmony with those of Grant H-1891 (C5) entitled "Precursors of Hypertension and Coronary Artery Disease" awarded by the National Heart Institute. The funds from that source do not include most of the items covered by the budget given above. Where similar items exist in each of the two budgets, it is because the budget from Grant H-1891 (C5) is insufficient to meet the total expense of a given item, and the two budgets will be used in such a way that they supplement each other.

Signature Caroline Bedell Thomas
Director of Project

Business Officer of the Institution

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Caroline B. Thomas, M.D.
The Johns Hopkins Sch. of Med.
Report #1
TIRC Grant #203
Part C. Plans for the Future.

CONFIDENTIAL

After July 1, 1958, our research program will be attached to the newly inaugurated Division of Medical Genetics within the Department of Medicine. A full-time statistician is to be appointed, whose services will be shared equally by Dr. Victor A. McKusick, Head of the new Division, and Dr. Caroline B. Thomas, for her studies of possible precursors of hypertension and coronary heart disease. Dr. Abraham Lilienfeld, an epidemiologist of highest caliber, is to have a dual appointment in the Division of Medical Genetics and the School of Hygiene and Public Health, where the appointee is to fill the newly created Professorship of Chronic Diseases. For the first time, therefore, an epidemiologist of stature will be available for close and continuing collaboration in planning our future work and follow-up studies. Also, there are to be several trainees in the field of Medical Genetics, one or more of whom may assist Dr. Thomas' studies. Finally, Mrs. Bernice H. Cohen, who analyzed the data for our studies of familial hypertension and coronary disease, is to receive her Ph.D. degree in Human Genetics under Dr. Bentley Glass at the Johns Hopkins School of Medicine in June, 1958 and will be associated with Dr. Thomas and Dr. McKusick after September, 1958. Her thesis has to do with the relationship of ABO and Rh blood groups to selective fetal loss, and she has done other work along these lines which makes her something of a specialist in regard to the genetics of blood groups.

Circumstances are, therefore, extremely favorable for vigorous inquiry into the following question:

Do smokers and nonsmokers have demonstrable genetic differences?

Our recently published family studies described in Part A and Appendix I suggest that genetic differences do, in fact, exist. This is an extremely important point from the public health standpoint, in view of studies indicating that heavy smokers have higher death rates from cancer of the lung and coronary disease than nonsmokers, and every effort should be made to establish the truth of the matter by the use of as many genetic indicators as possible. On a recent visit to Baltimore, Sir Ronald Fisher, eminent British statistician, discussed the problem and suggested that analysis of the various blood groups, of which there are now some nine, plus other "markers," might settle the matter. He stated that satisfactory proof of genetic differences would be at hand if one or more blood groups occurred in significantly different proportions among smokers and nonsmokers. Other human genetic indicators of which there are now more than twenty, could also be used in the same way to provide independent evidence.

In his recent report, Heath indicates that he did not find significant differences in the blood groups of 61 nonsmokers, 95 moderate and 96 heavier smokers selected from among Harvard undergraduates (Part A ref. 1). However, he did not study the Rh factor, he tells me, or other rarer blood groupings; his observations were confined to studies of the ABO group.

The proposed studies for September 1, 1958 -- August 31, 1959 are in two parts:

1. Studies of genetic differences between smokers and nonsmokers: cholesterol levels, Rh and ABO blood groups, other genetic indicators. This study would check our findings of positive relationships between smoking and higher cholesterol levels and between smoking and parental history in a larger and

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different population; obtain new information in regard to the Rh factor and other genetic indicators, and amplify Dr. Heath's observations on the ABO blood group, in view of the fact that the numbers in his series were small.

- a. Subjects: blood bank donors at the Johns Hopkins Hospital. There are around 10,000 healthy donors per year; Rh and ABO blood groupings are carried out on them all routinely, and a brief medical history is obtained.
- b. Additional data to be gathered: smoking habits and parental history on all possible donors; serum cholesterol and other genetic indicators on statistically suitable groups.
- c. Collaborators: Dr. McKusick, Dr. Lilienfeld and Mrs. Cohen.
- d. Statistical design: details to be determined after Dr. Lilienfeld and Mrs. Cohen join the Division of Medical Genetics.
- e. Needs:
 - (1) statistical clerk
 - (2) service fees for laboratory tests
 - (3) office supplies, reprints, etc.

2. Studies of psychological differences between smokers and nonsmokers as shown by comparison of figure drawings.

The use of figure drawings provides a new approach to the understanding of differences in the personality of smokers and nonsmokers. This projective technique has attracted much interest as a clinical tool, and is beginning to be used in a quantitative way to characterize contrasting groups of subjects (1-5). Over 600 Johns Hopkins medical students (classes of 1952 through 1961) have completed figure drawing tests. These have been analyzed by Dr. Edward Slockbower, the Psychologist associated with our study, who is outstanding in his field. In the next two years, it is our intention to classify the figure drawing material from many points of view, making cross correlations with our extensive genetic, physiological and psychological data. It is thought that a broad classification of figure drawings can be developed from our multifaceted studies which will be of great assistance to others working with healthy young adults of superior intelligence. At present no such unified classification of "normal" human figure drawings exists.

In view of the finding by Heath, using a combined questionnaire and interview method, of statistically significant personality differences between smokers and nonsmokers, (Part A, ref. 1) efforts should be increased to discover valid objective differences through the use of psychological tests. Preliminary studies along these lines using the Rorschach test were not very encouraging, but are being continued (see Part A). The figure drawing test is an independent projective method. It should be possible to determine with confidence whether or not quantitative differences in the figure drawings of smokers and nonsmokers exist by:

- a. direct measurement, counting and comparison of the drawings themselves (size, activity and position of figures, clothing or lack of clothing, proportion of transparencies, stick figures, omission of parts, etc.)
- b. comparison of the frequency of attributes described by Dr. Slockbower

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in his analyses of the figure drawings without any knowledge on his part of the smoking habits of the subjects studied.

- c. identification and measurement of a number of "indicators" described in the literature as of statistical importance in certain comparisons: younger people versus older people, for example.

These studies then, will be planned as follows:

- d. Subjects: over 600 Johns Hopkins medical students
- e. Additional data to be gathered: accurate objective description and measurement of over 1200 figure drawings (each subject draws a man and a woman).
- f. Collaborators: Dr. Edward Slockbower, Dr. Mary Ainsworth, Associate Professor of Psychology, the Johns Hopkins University.
- g. Statistical design: in general, the plan will be along the lines described above, with the assistance of our collaborators and statistician as the project actually starts and thereafter.
- h. Needs:
 - (1) statistical clerk
 - (2) office supplies
 - (3) funds for reproducing figure drawings by Xerox method

References

1. Levy, S.: L.E. Abt and L. Bellak (Eds.), Projective Psychology. N.Y.: Knopf, 1950, pp. 257-297.
2. Lorge, I., Tuckman, J., and Dunn, M.B., Am. Psychologist, 9:420, 1954.
3. Silverstein, A.B. and Robinson, H.A., J. Con. Psychology, 20:333, 1956.
4. Reznikoff, M. and Tomblen, D., J. Con. Psychology, 20:467, 1956.
5. Lakin, M., J. Con. Psychology, 20:471, 1956

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Itemized Annual Budget

September 1, 1958 - August 31, 1959

Salaries:		\$5,500.00
A. Director	\$2,000.	
B. Statistical clerks (two, part-time)	3,500.	
Expendable Supplies		222.25
Permanent Equipment		---
Fees for service: cholesterol determinations, other genetic indicators, reproduction of figure drawings, reprints, etc.		4,200.00
Social Security (2.25% of \$3,500* (est.))		<u>78.75</u>
Net appropriation		10,000.00
Overhead (15% of direct costs, based on actual expenditures (est.))		<u>1,500.00</u>
Total appropriation		\$11,500.00

*Estimate does not include SS. Tax from item A, as this S.S. Tax has already been deducted from another budget.

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Progress Note September 1, 1958 - April 30, 1959

Tobacco Industry Research Committee Grant

A. Studies of Genetic Differences

Using the interview technique, we are classifying healthy blood donors coming to the Johns Hopkins Hospital Blood Bank as to their smoking habits and parental history of coronary heart disease, hypertension and cancer. More than three-quarters of these donors are under the age of 40, and over half are white men. All the interviews have been carried out either by Dr. Bernice H. Cohen, an expert in human genetics who is collaborating with me in this study, or by Mrs. Mary Brumfield, a trained field worker and interviewer.

So far, 1105 donors have been registered in the study. Information concerning ABO and Rh blood groups, STS and blood pressure are obtained from the Johns Hopkins Blood Bank. In addition to the questions on smoking habits and parental history, the interviewers have administered a phenylthiocarbamide taste test to each donor, since differences in response to this test are genetically determined.

Month	Donors Studied according to Race and Sex									
	WM		CN		WF		CF		TOTAL	
	No	%	No	%	No	%	No	%	No	%
October	82	72.6	18	15.9	11	9.7	2	1.7	113	99.9
November	100	58.1	47	27.1	20	11.4	6	3.4	173	100
December	112	63.3	44	24.8	18	10.2	3	1.6	177	99.9
January	130	56.7	59	25.7	36	15.7	4	1.7	229	99.8
February	101	63.5	35	22.1	15	9.4	8	5.0	159	100
March	126	70.8	29	16.3	18	10.1	5	2.8	178	100
April	47	61.8	20	26.3	8	10.5	1	1.3	76	99.9
Total	698	63.2	252	22.8	126	11.4	29	2.5	1105	99.9

Determinations of total serum cholesterol have been carried out on 670 of the white male donors to compare with our findings in the Johns Hopkins medical students. It will be recalled that we found significantly more smokers with high cholesterol levels than nonsmokers among the medical students. It is important that this finding be evaluated again in an independent sample of healthy young white men.

B. Studies of Psychological Differences between Smokers and Nonsmokers as Shown by Comparison of Figure Drawings.

A preliminary study of the characteristics of the figure drawings of regular cigarette smokers and of nonsmokers is in progress with the collaboration of Dr. Irvin Greenberg. Using the records of white male subjects only, the figure drawings of all heavy cigarette smokers tested by Dr. Slockbower were divided into two equal groups of 24 subjects each. Drawings by an equal number of nonsmokers tested by Dr. Slockbower were selected at random, so that, in all, four groups of drawings by 24 subjects per group were formed. A set of drawings by smokers and one by nonsmokers was given to Dr. Greenberg without his knowing which set was drawn by which. Accordingly, this is a blind experiment. He is comparing the prevalence of nearly a score of variables in the drawings of smokers versus nonsmokers. Wherever significant differences are found, the same comparison is being made in the second set of smoker-nonsmoker groups.

We also have devised a code for the classification of figure drawings and are in the process of putting the characteristics of approximately 750 sets of figure drawings on IBM cards.

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DATE: _____ INTERVIEWER: _____ NO. _____
 NAME: _____ AGE: _____ MALE ☐ FEMALE ☐ U.H.NO. _____
 ADDRESS: _____ WHITE ☐ NEGRO ☐ OTHER ☐ STUDY NO. _____

BP _____	COMMENTS: _____
PTC _____	
ABO _____	
RH _____	
STS _____	
CHOLESTEROL _____	REL. GIVING BLOOD: _____
	OCCUPATION _____

SMOKING HABITS: Fill in the box that applies to you (A, B or C)

<p>A. I smoke <input type="checkbox"/></p> <p>daily <input type="checkbox"/> occasionally <input type="checkbox"/></p> <p>age begun _____</p> <p>total years of smoking _____</p> <p>How many do you smoke per day? _____</p> <p>Cigarettes: _____</p> <p>Cigars: _____</p> <p>Pipes: _____</p> <p>Do you inhale? yes/no _____</p>	<p>B. I used to smoke <input type="checkbox"/></p> <p>daily <input type="checkbox"/> occasionally <input type="checkbox"/></p> <p>age begun _____ age stopped _____</p> <p>total years of smoking _____</p> <p>How many did you smoke per day? _____</p> <p>Cigarettes: _____</p> <p>Cigars: _____</p> <p>Pipes: _____</p> <p>Did you inhale? yes/no _____</p>	<p>C. I do not smoke <input type="checkbox"/></p> <p>and never <input type="checkbox"/></p> <p>have _____</p> <p>except a few _____</p> <p>times years ago <input type="checkbox"/></p>
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PARENT'S MEDICAL HISTORY (Fill in boxes for both parents)

	FATHER:				MOTHER:			
Smoking habits: Kind	cigarettes/pipes/cigars/other				cigarettes/pipes/cigars/other			
Amount	regular/occasional/former/none				regular/occasional/former/none			
Age	at present _____ at death _____				at present _____ at death _____			
Cause of death								
Was death sudden	yes <input type="checkbox"/> no <input type="checkbox"/>				yes <input type="checkbox"/> no <input type="checkbox"/>			
Did parent ever have: (✓yes, no or ?/U for each)	Yes	No	*/U	Age or ages	Yes	No	*/U	Age or ages
High blood pressure								
Stroke(s)				(first found)				(first found)
Heart attack(s)								
Other heart trouble								
Cancer								
Cancer of lung								
Health at present								

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Name: _____

Please check one:

I smoke every day _____

☒ I smoke sometimes _____

I use to smoke but not now _____

I do not smoke and never have _____

I have smoked: (Check one or more) _____ (Write number)

cigarettes _____

Number cigarettes smoked each day _____

cigars _____

Number cigars smoked each day _____

pipes _____

Number pipes smoked each day _____

none of these _____

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Blood Bank No. _____	PLEASE DO NOT WRITE ON THIS SIDE
ABO _____	
RH _____	
Name _____	
Address _____	
Date _____	
S _____ R _____	
Age _____	

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June 1, 1958 - May 31, 1959

Progress Report for Research Grant H-1891 (C4)

During the current year, we have continued to collect basic data on successive classes of Johns Hopkins medical students, to pursue our follow-up studies, to make intensive investigations of certain aspects of our general studies and to analyze and report the results thus far obtained. Specifically, our activities may be summarized as follows:

A. Collection of basic data.

1. In collaboration with the Personnel Health Clinic, admission physical examinations, urinalyses, teleoroentgenograms, serological tests for syphilis and serum cholesterol levels were all obtained on the entering class of 1962.
2. The class of 1962 is now coming into our laboratory by individual appointment for their initial studies. A battery of psychological tests, including a group Rorschach test, figure drawing test and Strong Vocational Interest Schedule is to be carried out on them this week.
3. Serum cholesterol levels were measured this fall on members of the class of 1961, just a year after their entrance to medical school.
4. Members of the class of 1961 have come in by individual appointment for ballistocardiographic smoking tests; these are nearly complete.
5. The class of 1959 has been instructed how to obtain detailed information for the "long form" family history questionnaire in collaboration with parents and home physician. When this is complete, the subject comes in for final interview by appointment. At that time, another cholesterol level is obtained as well as blood pressure, heart rate and body weight.

B. Follow-up studies.

1. The five year follow-up is being carried out on the class of 1954 by means of a questionnaire.
2. The ten year follow-up is being carried out in similar fashion on the class of 1949, the second class thus studied.

C. Special investigations.

1. Further studies on the effect of Vitamin B₁₂ on cholesterol level are in progress to check our preliminary findings of last year which appeared to show that Vitamin B₁₂ administered orally lowers cholesterol levels in healthy young adults. A double blind experiment is being carried out on eight pairs of

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subjects from the class of 1961 matched as to cholesterol level and on one additional hypercholesteremic subject. Four control cholesterol determinations based on duplicate serum samples and an equal number of blood Vitamin B₁₂ determinations were obtained on each subject in December and January. During February and March, one member of each pair received Vitamin B₁₂ orally in the form of "Visorbin", while the other member received a placebo. During April and May, the group first given Vitamin B₁₂ is receiving the placebo, while the original group is now on Vitamin B₁₂. Both substances are supplied us through the kindness of Smith, Kline and French. The Vitamin B₁₂ determinations are carried out without charge in Dr. Bacon Chow's laboratory. Cholesterol levels and blood Vitamin B₁₂ levels are obtained weekly.

2. Because our studies of the medical students raised the possibility of seasonal variation of cholesterol levels, we are studying the effect of time of year on the cholesterol level of a group of healthy young adults in Baltimore who presumably live under more uniform conditions than the medical students as to periods of stress (examinations) on the one hand, and relaxation (vacations) on the other. The subjects for this study are 25 white male prisoners aged 20-29 years in the Maryland Penitentiary, which is less than a mile from the medical school. Monthly cholesterol determinations on each subject were begun in the fall and will continue for a year. All of the prisoners selected had been confined for more than a year at the start of the experiment and none were scheduled to be released within a year of it's termination.
3. This year we have made a systematic, detailed classification of the pairs of figure drawings produced by over 750 medical students as part of our psychological testing program. To our knowledge, no such classification has been described by others.
4. Under a grant from the Tobacco Industry Research Committee, we are comparing the figure drawings of smokers versus nonsmokers by this method. A preliminary comparison of the drawings of 24 smokers and 24 nonsmokers was made blind by Dr. Irvin Greenberg. Where differences were found, the drawings of 48 additional subjects, also equally divided as to being smokers or nonsmokers, were examined.
5. We are making further observations to answer the question: do smokers and nonsmokers have genetic differences? A survey of healthy blood donors (excluding medical students) is in progress, supported by the Tobacco Industry Research Committee. This study is to serve as an independent check of our previously reported findings based on studies of the medical students:
 - a. that a greater proportion of smokers than nonsmokers have high cholesterol levels.
 - b. that smokers more frequently have a positive parental history of hypertension and/or coronary heart disease than do nonsmokers.

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Dr. Bernice Cohen, an expert in the field of human genetics, is associated with me in this study. Blood donors are interviewed concerning smoking habits and parental history of hypertension and/or coronary heart disease. The Rh and ABO blood groupings as determined at the Johns Hopkins Blood Bank are recorded, and the donors are classified as "tasters" or "nontasters" of phenylthiocarbamide, a genetic marker. A cholesterol level is obtained on white male donors only for comparison with our findings on white male medical students.

D. Analysis of collected data.

1. We have continued to transfer the data for the classes of 1948-1958 to IBM punch cards. So far, eleven such cards per individual have been completed which include the following data: identification and general information; family history; medical history; blood pressure and heart rate under different circumstances; cholesterol levels; ballistocardiographic smoking test; smoking habits and four-way grouping; habits of nervous tension; Rorschach test; high lights of habit survey; sodium withdrawal studies and eosinophil counts. In the process of completion are cards concerned with the oximeter-controlled anoxemia test; figure drawings; life tables for parents of subjects.
2. In December, 1958, we were fortunate in receiving a supplementary grant from the National Heart Institute to cover the salary of a full-time statistician for two years to collaborate with the director of the study in writing a monograph on the collected data. With the help of Dr. Abraham Lillienfeld, Professor and Head of the Division of Chronic Disease in the School of Hygiene and Public Health, we have conducted an intensive search to find a well-qualified person. Several candidates have been interviewed and we hope to fill the position shortly.

E. Reporting of results.

1. Three talks based on our findings have been given:
 - a. On October 24, 1958, a paper entitled "Familial Patterns in Hypertension and Coronary Heart Disease" was presented as part of a symposium on genetic factors in cardiovascular disease at the 31st Scientific Sessions of the American Heart Association in San Francisco, California.
 - b. On November 21, 1958, a paper entitled "Hypertension and Humanity" was presented at the Annual Fall Conference of the Council for High Blood Pressure Research of the American Heart Association in Cleveland, Ohio.
 - c. On February 18, 1959, a discussion of our studies on the precursors of hypertension and coronary disease

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was presented at a staff seminar at the Howard University School of Medicine in Washington, D.C.

2. Three papers have been published:

- a. Thomas, C.B. and Murphy, E.A.: The Circulatory Response to Smoking. J. Chr. Dis. 8:202, 1958.
- b. Thomas, C.B. and Murphy, E.A.: Further Studies on Cholesterol Levels in the Johns Hopkins Medical Students: The Effect of Stress at Examinations. J. Chr. Dis. 8:661, 1958.
- c. Thomas, C.B.: Cholesterol Characteristics. Maryland State Med. J., 8:2, 1959.

3. Two papers are in press and will appear shortly:

- a. Thomas, C.B. and Murphy, E.A.: Observations on Some Possible Precursors of Essential Hypertension and Coronary Artery Disease: VI. Comparison of the Circulatory Reactivity to the Cold Pressor Test and to the Smoking Test, to appear in Ann. Int. Med., Apr., 1959.
- b. Thomas, C.B.: Familial Patterns in Hypertension and Coronary Heart Disease, to appear in Circulation, May, 1959.

4. One paper has recently been submitted for publication:

- a. Thomas, C.B. and Eisenberg, F.F.: Variability of Cholesterol Levels in Individual Johns Hopkins Medical Students, with Observations on Stopping Smoking, Vitamin B₁₂ Administration and Acute Infection.

5. Three papers are in the process of completion:

- a. Thomas, C.B. Comparison of Healthy Young Smokers and Nonsmokers as to Parental History and Individual Characteristics.
- b. Thomas, C.B. and Murphy, E.A.: The Effect of Minimal Doses of Hexamethonium Chloride and Wyamine Sulphate on the Circulatory Response to Smoking.
- c. Garn, S.M. and Thomas, C.B.: Degree of Obesity and Serum Cholesterol Level.

6. An application has been made to the Heart Association of Maryland for funds to support the republication in a single volume of the first twenty-five papers arising from this study (including those listed under 2 and 3 above).

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Application For Research Grant

#203R1

cf. #89

activated 10/1/55

renewed 7/1/56

#168

activated 9/1/57

#203

activated 9/1/58

Date: May 11, 1959

1. Name of Investigator: Dr. Caroline Bedell Thomas
2. Title: Associate Professor of Medicine
3. Institution & Address: The Johns Hopkins School of Medicine
710 North Washington Street
Baltimore 5, Maryland
4. Project or Subject:
 - a. Studies of Genetic Differences between Smokers and Nonsmokers.
 - b. Studies of Psychological Differences between Smokers and Nonsmokers as Shown by Comparison of Figure Drawings.
5. Detailed Plan of Procedure:

The background for our future plans may be found in Attachments I, II and III. Attachment I gives the original plan for the two year study as presented in our application to the TIRC in 1958. Attachment II indicates briefly the progress made in each phase of the present studies this year. Attachment III summarizes progress made in 1958-1959 in our over-all program and lists the publications. In III, note particularly sections A4; C2, 3, 4 and 5; E2a and b, 3a, 4a, and 5 a and b. The paper listed in 3a has now been published, but reprints have not yet been received. Work is in progress on 5a and b; it is hoped that these papers will both be submitted for publication before September, 1959. Reprints for the papers listed under 2a and b are attached. The latter work was not primarily supported by the TIRC nor did Dr. Murphy (the part-time Fellow supported by the TIRC) have any part in the design or execution of the experiment or in the writing of the paper per se. His contribution was to make a statistical analysis of the data in Table III at a time when other statistical assistance was not available.

Plans for the coming year:

A. Studies of Genetic Differences between Smokers and Nonsmokers.

1. to continue the orderly collection of data from blood donors as has been done this year (see Attachment II).
2. to try out taking smoking histories by a self-administered questionnaire (see card at end of Attachment II) rather than by interview. If this proves satisfactory, the data on blood groups in smokers versus nonsmokers can be expanded to include many more donors than it is possible to interview. (Our interviewers are at the Blood Bank about 4 hours a day five days a week.)

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3. to make a preliminary analysis of the data thus far collected in the near future in order to obtain an estimate of the total number of subjects it will be desirable to include in the entire study.
 4. when data collection has been completed, to make a statistical analysis of the results and write them up.
- B. Studies of the Psychological Differences between Smokers and Nonsmokers as Shown by Comparison of Figure Drawings.
1. to complete the preliminary study with Dr. Greenberg described in Attachment IIB.
 2. to make a statistical analysis of the smoker-nonsmoker comparisons for the variables coded on IBM cards (see Attachment IIB).
 3. to incorporate the results of these two studies into one or two papers.

6. Budget Plan:

Salaries	5,500.00
Expendable Supplies	222.25
Permanent Equipment	
Overhead (15%)	1,500.00
Other	4,278.75
Total	\$11,500.00

7. Anticipated Duration of Work: one year.

8. Facilities and Staff Available: Dr. C. Lockard Conley and Dr. Julius R. Krevans, who are in charge of the blood bank have given us permission to make use of blood bank donors and blood group data in the manner indicated. There is ample work space available in conjunction with our major project. During the next two years, we shall have a full-time statistician working with us. A part-time psychologist is already on our staff.

9. Additional Requirements: none.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The aims of the project outlined above are in harmony with those of Grant H-1891 (C5) entitled "Precursors of Hypertension and Coronary Artery Disease" awarded by the National Heart Institute. The funds from that source do not include most of the items covered by the budget given above. Where similar items exist in each of the two budgets, it is because the budget from Grant H-1891 (C5) is insufficient to meet the total expense of a given item, and the two budgets will be used in such a way that they supplement each other.

Signature Caroline Bedell Thomas
Director of Project

Business Officer of the Institution

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TIRC Grant
#203

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Vol. 8, No. 6, Pages 661-668, December, 1958 (Printed in the U. S. A.)

FURTHER STUDIES ON CHOLESTEROL LEVELS IN THE JOHNS HOPKINS MEDICAL STUDENTS: THE EFFECT OF STRESS AT EXAMINATIONS

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(Received for publication Sept. 5, 1958)

THE variability of serum cholesterol levels found in healthy subjects on repeated determinations depends on many factors.^{1,2} When the method has been carefully standardized and the technical error of measurement is small, there are still wide variations in the cholesterol level of a given individual, occurring both from week to week and from year to year, which cannot be attributed entirely to age or to diet.^{1,3} The influence of various forms of stress on the cholesterol level is of increasing interest and importance. A number of investigators have found that cholesterol levels are appreciably higher during periods of stress than at other times.⁴⁻⁹ While the exact significance of this finding is not yet clear, the possibility exists that such elevations of cholesterol level may contribute to the early onset of coronary artery disease in some persons.

As part of a long-term study on possible precursors of hypertension and coronary disease, we have made continuing studies of the cholesterol levels of successive classes of the Johns Hopkins medical students. The class of 1961, entering in the fall of 1957, was the fourteenth class registered in the study. It was decided to obtain cholesterol determinations on that class during the final anatomy examination period to compare with other levels obtained during the

This study was supported in part by Research Grant H-1891, National Heart Institute, and in part by the Tobacco Industry Research Committee.

same year, since it is generally considered that the final anatomy examination is a severe form of stress, probably the greatest encountered by the medical students as a whole during their four academic years.

METHOD

The class of 1961 was examined in the different branches of anatomy over a period of 5 days in January, 1958. Each student had oral examinations on 3 successive days followed by 2 days of practical examinations. Through the kind cooperation of Dr. David Bodian, Professor of Anatomy, the students were asked to come to the laboratory for stress studies immediately after one of these examinations. Body weight, blood pressure, and heart rate were measured, then blood was drawn for a blind duplicate cholesterol determination and a circulating eosinophil count. Data on the same subjects were also obtained at the entrance physical examination carried out in the autumn by members of the staff of the Department of Medicine and also when, at a varying interval after the anatomy examination period, the subject came to our laboratory for research purposes by individual appointment. Total serum cholesterol was determined in the Clinical Chemistry Laboratory of The Johns Hopkins Hospital by the Buell modification of the Bloor method as previously described.¹¹ Total circulating eosinophils were counted by the method of Hills, Forsham, and Finch.¹⁰

The three tests are identified in chronologic order by Roman numerals. Details concerning the collection of data at each test are shown in Table I. Although there were minor differences in the frames of reference, none are of much importance except that blood pressure and heart rate were measured sitting in Test II and recumbent in Tests I and III. Time of day was similar for Tests II and III, so that the eosinophil counts are quite comparable in that respect.

TABLE I

TEST	OCCASION	DATE	WEIGHT	BLOOD PRESSURE AND HEART RATE	NUMBER OF SERUM CHOLESTEROL SAMPLES	EOSINOPHIL COUNT
I	Admission physical examination	Oct. 1-3, 1957	In shirt, trousers, and shoes	Initial, recumbent	1	None obtained
II	Anatomy examination	Jan. 6-10, 1958	Same as I	Initial, sitting	2	Direct count
III	Laboratory studies	Jan. 21-April 24, 1958	In trousers and shoes	Initial, recumbent	2	Direct count

For the sake of consistency, the statistical analysis in this report is based on the protocols of the 52 men with complete cholesterol data. The 17 remaining men in the class of 1961 were not included for the reasons shown in Table II. The 5 women were excluded because of reports of fluctuation of cholesterol level with the menstrual cycle, which would introduce another biologic variable.^{11,12}

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TABLE II

REASON	NUMBER
Did not report for Test II	11*
Originally in the class of 1960; had Test III the previous year	2
One determination only at Test II	2
One determination only at Test III	2

*Two of these men had stress studies at the time of the final biochemistry examination; their findings are presented separately, but are not included in the group data.

The 52 men studied were 19 through 26 years of age; three were 19 to 20, four were 24 to 26, and the rest were 21 to 23 years old. All were in good health. None had hypertension, coronary disease, diabetes, nephrosis, thyroid disease, or any other disorder thought to influence cholesterol level. There were no pronounced variations in habits of exercise, diet, or smoking between Tests II and III. For many subjects, exercise habits and diet were undoubtedly different during the summer than after entering medical school, so that the conditions preceding Test I, carried out 16 to 18 days after admission, were less consistent in these respects. These studies could not be directly controlled as to possible seasonal variations in cholesterol level in the absence of stress, since all medical students undergo stressful situations periodically. A study of seasonal cholesterol values among institutionalized patients or prisoners in this locality might provide helpful information in this regard.

RESULTS

The cholesterol levels of the 52 male medical students at final anatomy examination and at two other times of year are shown in Table III. It will be seen that the highest mean cholesterol value (225.7 mg. per 100 c.c.) was present at the time of the anatomy examination and the mean level of 204.7 mg. per 100 c.c. at Test III was significantly lower ($P < 0.001$). However, there was no significant difference between the mean level of 224.4 mg. per 100 c.c. at Test I, carried out 16 to 18 days after entering medical school, and that of Test II. Following Test II, 39 subjects exhibited a fall of 67.5 to 7.5 mg. per 100 c.c. in cholesterol level, averaging 31.4 mg. per 100 c.c.; four showed no change, and nine subjects showed a rise of 4.0 to 28.5 mg. per 100 c.c., averaging 14.8 mg. per 100 c.c. It is noteworthy that two-thirds of the group showing a rise had cholesterol levels under 200 mg. per 100 c.c. both at Test II and Test III, while less than one-quarter of those with a fall in cholesterol after Test II showed a similar low range at both tests. Two subjects had hypercholesteremic levels* at Test II but not at Test III. Hypercholesteremia was also present at Test I in Subject 61114 but not in Subject 61156. In these two subjects, the fall in cholesterol between Tests II and III of 40 and 67 mg. per 100 c.c., respectively, is somewhat greater than the mean for the group of 39 as a whole.

*Cholesterol of 300 mg. per 100 c.c. or more.

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TABLE III. CHOLESTEROL LEVELS OF 52 MALE MEDICAL STUDENTS AT ANATOMY EXAMINATION COMPARED WITH TWO OTHER TIMES OF YEAR

CHOLESTEROL LEVEL (MG. PER 100 C.C.)						
	I	II	III	II-I	III-II	III-I
Mean	224.4	225.7	204.7	1.3	-21.0	-19.6
S.E. mean	± 5.396	± 5.539	± 4.311	± 4.394	± 3.367	± 3.835
Median	221.5	229.0	202.2	2.2	-21.0	19.0
t				0.302	6.225****	5.120****
Direction of change	+			27	9	12
	0			0	4	0
	-			25	39	40

I = At admission physical examination Oct. 1-3, 1957.

II = At final anatomy examination Jan. 6-10, 1958.

III = At our laboratory later in 1958 by individual appointment.

****P < 0.001.

TABLE IV

SUBJECT NUMBER	AGE	SAMPLE NUMBER	CHOLESTEROL (MG. PER 100 C.C.)		
			TEST I	TEST II	TEST III
61114	23	1	325	305	270
		2	—	315	270
		Mean	—	310	270
61156	23	1	287	305	248
		2	—	325	248
		Mean	—	315	248

Since the mean cholesterol level for the entire group fell significantly between Tests II and III, the data were examined to determine whether or not a positive association existed between the degree of lowering of cholesterol and the interval of time between Tests II and III. Test III was carried out in random sequence on the 52 subjects 13 to 105 days after Test II. Both the interval between the two tests and the fall in cholesterol between Tests II and III had fairly normal distributions.

The over-all correlation coefficient between fall in cholesterol and interval between Tests II and III was not found to be significant ($r = 0.049$). However, when these two variables were plotted in a scatter diagram, the shadow of the effect of the final biochemistry examination appears to be imprinted there (Fig. 1). The final biochemistry examination fell on March 19, 68 days after the end of the anatomy examination period. For the 16 days prior to the biochemistry examination, no fall in cholesterol level as great as 25 mg. per 100 c.c. was found,

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and not until 10 days after that examination were such falls again present. It is also noteworthy that no fall of great magnitude was observed in the first 3 weeks after the anatomy examination.

The cholesterol levels of the two subjects who had stress studies at the biochemistry examination rather than at the anatomy examination are pertinent here.* Their data are not included in Table III or Fig. 1. Chronologically the time relationship of the biochemistry stress studies to Test III was just the opposite of the anatomy stress studies, that is, the stress studies followed Test III rather than preceded it. The change in cholesterol level paralleled this reversal. Rises of 30 and 25 mg. per 100 c.c., respectively, were recorded between Test III and the biochemistry examination. Thus it appears that higher cholesterol values were associated with both the anatomy and the biochemistry examinations and lower cholesterol values were more frequently found at periods of time beginning 2 or 3 weeks after the examination in question was over.

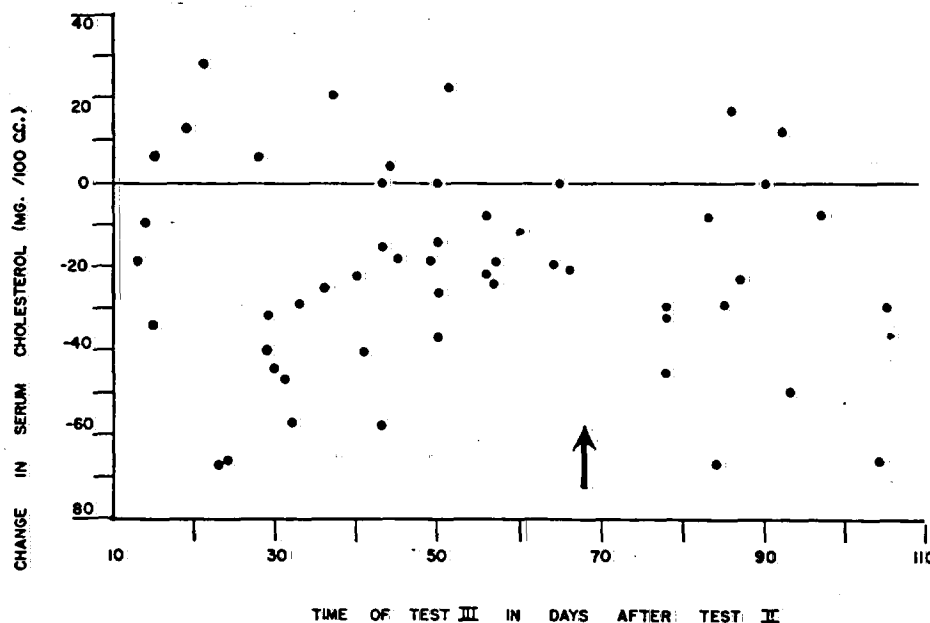


Fig. 1.—The relationship of the change in cholesterol level to the interval between the anatomy examination (Test II) and Test III among 52 Johns Hopkins students. The change in cholesterol level expressed in milligrams per 100 c.c. Each dot represents the absolute change for a single subject. Test II was carried out at final anatomy examination. Test III was performed in our laboratory by individual appointment for research purposes only 13 to 105 days after Test II.

↑ Indicates March 19, 1958, the day of the final biochemistry examination.

That the change in cholesterol level between Tests II and III did not depend on change in body weight is demonstrated in Table VI. Mean body weight decreased about one pound between Tests I and II while cholesterol remained stable but did not change between Tests II and III while cholesterol decreased. The small weight change between Tests I and II was not statistically significant.

*These two men, the only ones of the 11 absentees at Test II who came on request for Test IV, happened to have astonishingly similar data.

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TABLE V

SUBJECT	SAMPLE	TEST I	TEST II (ANATOMY)	TEST III	TEST IV (BIOCHEMISTRY)
61104	1	Oct. 3, 1957	Jan. 6-10, 1958	Jan. 23, 1958	March 19, 1958
		200	—	165	200
	2	—	—	180	205
	Mean	—	—	172.5	202.5
61136	1	Oct. 1, 1957	—	Feb. 27, 1958	March 19, 1958
		200	—	180	200
	2	—	—	175	205
	Mean	—	—	177.5	202.5

TABLE VI. BODY WEIGHT, CIRCULATING EOSINOPHILS, BLOOD PRESSURE, AND HEART RATE AT ANATOMY EXAMINATION (II) COMPARED WITH TWO OTHER TIMES OF YEAR

MEASUREMENT	N		I	II	III	II-I	III-II	III-I
Body weight	52	Mean	172.1	171.0	171.0	-1.1	0.0	-1.2
		S.E. mean t	±2.93	±3.19	±3.11	0.74 1.52	±0.25 0.08	±0.74 1.58
Total circulating eosinophils (c.mm.)	47	Mean	—	97.0	129.1	—	32.1	—
		S.E. mean t	—	±9.45	14.53	—	±11.06 2.90**	—
Systolic pressure	52	Mean	122.4	128.7	122.7	6.2	-5.9	0.3
		S.E. mean t	±1.63	±1.49	±1.22	±2.05 3.04***	±1.88 3.16***	±1.83 0.16
Diastolic pressure	52	Mean	72.5	78.7	64.1	6.2	-14.7	-8.5
		S.E. mean t	±1.23	±1.42	±1.21	±1.76 3.53****	±1.20 12.19****	±1.53 5.52****
Heart rate	52	Mean	77.4	81.0	75.0	3.6	-6.0	-2.4
		S.E. mean t	±1.57	±1.97	±1.61	±1.86 1.92	±2.05 2.94***	±1.51 1.61

** 0.01 > P > 0.005.

*** = 0.005 > P > 0.001.

**** = P < 0.001.

It is now well known that the number of circulating eosinophils in the peripheral blood falls during periods of stress of various kinds. Although eosinophil counts were not obtained at Test I, 47 of the 52 subjects under investigation had eosinophil counts both at Test II and Test III. Their mean eosinophil count at Test II, the anatomy examination, was 97 per c.mm., while at Test III it was 129 per c.mm. Assuming that the more usual values were present at

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Test III, a significant fall of 32 per c.mm., or nearly 25 per cent of the total circulating eosinophils, appears to have taken place at the time of the anatomy examination, with subsequent recovery.

In regard to the findings concerning blood pressure and heart rate, it should be recalled first that the subjects were sitting at Test II and recumbent at Tests I and III. With this distinction in mind, the significant differences indicated in Table IV, all but one of which involve comparison of Tests I or III with Test II, must be interpreted with caution. The finding that the systolic pressure at Test II is significantly higher than at Tests I or III, when the mean levels were almost identical, could result from the effect of posture alone without regard to stress. Both diastolic pressure and heart rate show a gradation in mean values, with the highest level at Test II and the lowest at Test III; Test I assumes an intermediate position. The three means are significantly different from each other for diastolic pressure only, but a similar trend exists in regard to heart rate. Although too much emphasis should not be placed on the elevations of diastolic pressure and heart rate at Tests II and I, they suggest that stress as well as posture may be playing a role.

DISCUSSION

We have presented statistical evidence showing that the mean cholesterol level was higher at the anatomy examination than at a later, less stressful time. This finding is consistent with the hypothesis that serum cholesterol levels rise during periods of stress. Moreover, the eosinophil data supports the view that the students as a group were actually experiencing more stress at Test II than at Test III. If, as seems most likely, the elevation of cholesterol encountered at Test II was indeed due to stress, then the similar elevation at Test I was probably the result of stress from a somewhat different cause. At Test I, the students were going through an intensive period of adaptation, having been in medical school less than 3 weeks. They were dissecting a human cadaver for the first time, in addition to becoming accustomed to new living conditions, new classmates, and new methods of teaching. This unexpected finding added to some evidence that the biochemistry examination was also accompanied by elevation of cholesterol indicates that situations stressful enough to make a significant difference in cholesterol level are not uncommon, at least in a medical student's life.

These findings are similar to those of Wertlake and his colleagues,⁹ whose report on 44 medical students appeared while this study was in progress. They found an 11 per cent rise in cholesterol from the control period to the stress period. Using Test III as our control period, our subjects showed a 10.3 per cent elevation of cholesterol at Test II and a 9.6 per cent elevation at Test I. The mechanism of this change, if indeed it be due to stress, remains to be explained.

SUMMARY

1. Serum cholesterol determinations on 52 male medical students were carried out at admission to medical school (Test I), at final anatomy examination (Test II), and during a period of regular academic work (Test III).

EST IV
HEMISTRY)

h 19, 1958

200
205

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h 19, 1958

200
205

202.5

T RATE AT

III-I
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±0.74
1.58
—
—
—
0.3
±1.83
0.16
-8.5
±1.53
5.52....
-2.4
±1.51
1.61

in the
ough eo-
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Test III
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2. The mean cholesterol levels at Tests I and II were significantly higher than at Test III.

3. The cholesterol levels of two additional subjects showed a similar pattern when the values at final biochemistry examination (Text IV) were compared with Test III.

4. For certain students, Test III fell close to the final biochemistry examination; the fall from Test II to Test III was less marked in these subjects.

5. The total eosinophil count was significantly lower at Test II than Test III.

6. There was no significant difference in body weight between Tests I, II, and III.

7. The findings are consistent with the hypothesis that stress such as accompanies the first few weeks of medical school or important final examinations is accompanied by a significant mean rise in cholesterol level.

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Grant #203

THE CIRCULATORY RESPONSE
TO SMOKING

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EDMUND A. MURPHY, M.D.
Baltimore, Md.

From the Department of Medicine, The Johns Hopkins
University School of Medicine

Reprinted from

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(Printed in the U. S. A.)

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THE CIRCULATORY RESPONSE TO SMOKING

The Variation in Ballistocardiographic Smoking Tests in Healthy Young Men

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EDMOND A. MURPHY, M.D.
BALTIMORE, MD.

*From the Department of Medicine, The Johns
Hopkins University School of Medicine*
(Received for publication April 17, 1958.)

DURING the course of a project to discover what characteristics in the individual are of value in recognizing susceptibility to hypertension and coronary heart disease, a study of the circulatory response to smoking in healthy young adults was reported, in 1956, by Thomas, Bateman, and Lindberg.¹ Various patterns of response were described, and the mean patterns of subjects grouped on the basis of sex, body weight, smoking habits, or family history were compared. The most striking differences found were those associated with family history. On the average, subjects with a history of parental hypertension appeared much more reactive than did those with normal parents, while subjects with a history of parental coronary disease showed less response of blood pressure, heart rate, and cardiac output than either of the other two groups. Since both hypertension and coronary disease are thought to depend in part on genetic factors, these varying patterns of circulatory response to smoking may stem from inherited constitutional differences. If so, they would provide early indications of liability to cardiovascular disease in later life.

It is clear that differences in the pattern of response to smoking arise from two main sources of variation—true “constitutional” differences between individuals, and variations in physiologic responsiveness of the individual from time to time. It is also clear that however valuable this test may be in distinguishing between different groups, it will be of little value in individual prognosis unless the former source of variation is much more important than the latter. Accordingly, studies have been undertaken (1) to measure the reproducibility of results under standard conditions, and (2) to ascertain what factors influence the results and therefore should be standardized if the maximum amount of information is to be derived from smoking tests.

This study was supported in part by the Tobacco Industry Research Committee, in part by Research Grant H-1891, National Heart Institute and in part by Research Contract V1001 M-2768, Veterans Administration.

MATERIALS AND METHOD

All the subjects were free from clinical, electrocardiographic, hypertension or coronary disease. They were all male smokers throughout. The data in analyzing the ballistocardiogram are described.¹

Three series of sm

Series O.—Number 24.86 \pm 0.27.

Of the 113 individuals, 69 were male smokers after excluding the females. These tests were performed. Two-thirds were performed, at least 1 hour

Series F.—Number (1) for “I” tests 23.10

Four smoking tests in the class of 1958, between individual consisted of a fast (A) and one before breakfast on a day in during that winter of by one of three assistants the same assistant.* smoking, without blood pressure measured, after which was then done in the afternoon the subject smoked tracings were recorded and attended morning for the second (B) between 12:00 noon

The two tests for the A test the hours since breakfast before his B test, but active for most of the test, he had been c

*We should like to inform many of the sm

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MATERIALS AND METHODS OF PROCEDURE

All the subjects were Johns Hopkins medical students in good health and free from clinical, electrocardiographic, and ballistocardiographic evidences of hypertension or coronary heart disease. They were of comparable age and were all male smokers. The same Technitrol table ballistocardiograph was used throughout. The details of the method used in performing the smoking test and in analyzing the ballistocardiographic measurements were as previously described.¹

Three series of smoking tests provided material for this study.

Series O.—Number of subjects: 69. Mean age in years at last birthday: 24.86 ± 0.27 .

Of the 113 individuals whose single smoking tests have already been reported, 69 were male smokers.¹ The data for these subjects are here freshly analyzed after excluding the female subjects and the nonsmokers for the sake of homogeneity. These tests were performed at various times of day from 9:30 A.M. to 5:20 P.M. Two-thirds were performed between 2:30 P.M. and 5:00 P.M. With rare exceptions, at least 1 hour had elapsed since the last meal.

Series F.—Number of subjects: 32. Mean age in years at last birthday: (1) for "I" tests 23.16 ± 0.27 , (2) for "II" tests 23.31 ± 0.26 .

Four smoking tests were performed on each of the 32 male smokers in the class of 1958, between October, 1955, and June, 1956. The four tests on each individual consisted of two tests on each of two days (I and II), one before breakfast (A) and one before lunch (B). The first pair of tests (IA and IB) was carried out on a day in the fall of 1955, the second pair (IIA and IIB) on a day during that winter or in the spring of 1956. All of the 128 tests were performed by one of three assistants; almost always, each pair of tests was carried out by the same assistant.* The subject came to the laboratory at 8:00 A.M. without smoking, without breakfast, and without drinking coffee. He was weighed and measured, after which he lay quietly on the ballistocardiograph. The A test was then done in accordance with the method previously described except that the subject smoked one cigarette instead of two, and no electrocardiographic tracings were recorded.¹ After the A test he breakfasted, smoked if he pleased, and attended morning classes as usual, returning to the laboratory before lunch for the second (B) test of the day. He was not, however, allowed to smoke between 12:00 noon and his B test which was performed at 1:00 P.M.

The two tests on the same day, then, differed in the following respects: (1) for the A test the subject had been fasting overnight, for the B test for the 4 hours since breakfast only; (2) the subject was allowed to smoke up to 1 hour before his B test, but not for 8 hours before his A test; and (3) he had been inactive for most of the previous 8 hours before the A test, whereas before his B test, he had been occupied with the usual second year academic activities.

*We should like to acknowledge the assistance of Dr. Amarilli Kassam, part-time fellow, who performed many of the smoking tests.

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The tests on the second day (IIA and IIB) were carried out as nearly as possible in the same manner as those on the first day.

Series E.—Number of subjects: 6. Mean age in years at last birthday: (1) at first test 23.33 ± 0.42 , (2) at last test 23.67 ± 0.33 .

Over a period of 9 weeks in the summer of 1956, each of 6 male smokers underwent a series of eight smoking tests performed as far as possible under circumstances identical for each individual and conducted by the same observer (EAM). On the 8 test days, each subject followed a uniform pattern of activity, took a uniform amount of exercise, smoked the same number of cigarettes at the same time of day, and ate the same types of meals at the same times of day. The tests were performed at a time of day constant within 5 minutes either way for each individual, and all between 2:00 and 5:00 P.M. (resembling the majority of studies in series O). Since the period of time over which the observations were made was brief, weather conditions were fairly constant. It will thus be seen that while there were differences *between* individuals, for any *one* individual, the circumstances were as nearly as possible absolutely uniform. The ballistocardiographic measurements were all made by the same two technical assistants working together.

Consideration of these three series shows a varying degree of standardization: (1) series O: in this series all subjects were healthy young male smokers; (2) series F: all the subjects were healthy young male smokers and the tests were performed at two standard times of day under two standard conditions of smoking and eating; (3) series E: all the subjects were healthy young male smokers, whose eating, smoking, and exercise habits were, within the individual, uniform and the observer and the time at which the test was performed unvaried. The weather conditions were also fairly constant.

Most of the statistical techniques used in these analyses are described in Snedecor's textbook.² The use of "t" tests has been restricted to groups of figures found to have a normal distribution. The meaning of asterisks to denote levels of significance is defined in the footnotes.

RESULTS

The Interindividual Range of Circulatory Response to Smoking: the Series of Single Smoking Tests in 69 Subjects (Series O).—The means and variances for the results obtained in the 69 single smoking tests (Series O) are shown in Table II. Despite the greater homogeneity of the present group as the result of discarding the nonsmokers and the women, the findings are essentially the same as those for the 113 individuals set forth in the first table of the preceding paper.¹ The main importance of the new calculations here given in Table I is for the purposes of comparison with groups E and F (see below).

Comparison of Variation Within the Individual and Between Individuals Under Different Standardized Fasting Conditions: the Series of Two Tests Before Breakfast and Before Lunch on Each of 32 Subjects (Series F).—The results of

TABLE I. ANALYSIS

MEASUREMENT
Systolic pressure Control Change Final
Diastolic pressure Control Change Final
Pulse pressure Control Change Final
Heart rate Control Change Final
Stroke volume Control Change Final
Cardiac output Control Change Final

These probabilities with the variation in

Probabilities are

* P < 0.05
** P < 0.01
*** P < 0.001

Abbreviations:

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millimeters, and cardiac

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TABLE I. ANALYSIS OF RESULTS OBTAINED IN 69 SINGLE SMOKING TESTS ON MALE SMOKERS

MEASUREMENT	MEAN	(S.D.) ²	S.D.	S.E.M.
Systolic pressure				
Control	114.2	92.66	9.63	1.16
Change	2.61***	36.48	6.04	0.73
Final	116.8	100.84	10.04	1.21
Diastolic pressure				
Control	71.6	49.44	7.03	0.85
Change	4.93***	24.19	4.92	0.59
Final	76.6	59.28	7.70	0.93
Pulse pressure				
Control	42.6	87.37	9.35	1.13
Change	-2.32**	45.04	6.71	0.81
Final	40.2	102.72	10.14	1.22
Heart rate				
Control	70.8	122.03	11.05	1.33
Change	7.50***	48.01	6.93	0.83
Final	78.3	131.18	11.45	1.38
Stroke volume				
Control	109.7	250.07	15.81	1.90
Change	-3.84***	43.91	6.63	0.80
Final	105.9	297.48	17.25	2.08
Cardiac output				
Control	7.69	1.585	1.259	0.152
Change	0.473***	0.559	0.748	0.090
Final	8.16	2.081	1.443	0.174

These probability values are based on "t" tests for significance of change on smoking as compared with the variation in the change on smoking.

Probabilities are indicated by asterisks as follows:

- * P < 0.05
- ** P < 0.01
- ***P < 0.001

Abbreviations: S.D. = standard deviation; S.E.M. = standard error of the mean.

Blood pressure is recorded throughout in mm. Hg, heart rate in beats per minute, stroke volume in milliliters, and cardiac output in liters per minute.

this series of 128 smoking tests on 32 subjects are summarized in Table II.* Smoking one cigarette produces, in each instance, a highly significant mean rise in systolic and diastolic pressure, heart rate, and cardiac output and a significant mean fall in stroke volume. In the main, the systolic and diastolic pressures rise in parallel so that the mean pulse pressure is little affected. In Test IIB, however, the pulse pressure falls by an amount which is just significant. This pattern is substantially the same as that in the O series; the higher probability of significance for the change in the pulse pressure in the latter is in part a reflection of the larger number of observations.

*Because one of the series of ballistocardiograms was technically unsatisfactory in each of two subjects, all the calculations derived therefrom (heart rate, stroke volume, and cardiac output) were discarded. Thus there are data on blood pressure in 32 subjects and on the cardiac measurements in 30.

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TABLE II. THE EFFECTS OF SMOKING UNDER DIFFERENT CONDITIONS IN THE FASTING STATE: FOUR TESTS ON EACH OF 32 MALE SMOKERS (SERIES F)

OBSERVATION	N	IA. THE FIRST TEST BEFORE BREAKFAST					IIA. THE SECOND TEST BEFORE BREAKFAST				
		CONTROL MEAN	CHANGE AFTER ONE CIGARETTE				CONTROL MEAN	CHANGE AFTER ONE CIGARETTE			
			MEAN	S.D.	S.E.M.	t		MEAN	S.D.	S.E.M.	t
Systolic pressure	32	113.2	+5.4	6.56	1.16	4.63***	112.9	+6.5	6.49	1.15	5.70***
Diastolic pressure	32	66.3	+5.1	5.49	0.97	5.21***	67.0	+6.0	5.63	1.00	5.90***
Pulse pressure	32	46.9	+0.3	4.54	0.80	0.39	45.9	+0.7	4.27	0.75	0.87
Heart rate	30	60.8	+13.3	10.3	1.88	7.09***	60.0	+13.3	12.1	2.20	6.06***
Stroke volume	30	109.4	-6.2	10.1	1.84	3.35**	110.6	-5.5	9.46	1.73	3.18**
Cardiac output	30	6.57	+1.03	0.99	0.18	5.72***	6.60	+1.10	1.26	0.23	4.76***
OBSERVATION	N	IB. THE FIRST TEST BEFORE LUNCH					IIB. THE SECOND TEST BEFORE LUNCH				
		CONTROL MEAN	MEAN	S.D.	S.E.M.	t	CONTROL MEAN	MEAN	S.D.	S.E.M.	t
Systolic pressure	32	115.0	+2.4	3.22	0.57	4.28***	115.5	+4.3	5.23	0.93	4.70***
Diastolic pressure	32	67.0	+3.5	4.20	0.74	4.67***	66.9	+6.0	5.05	0.89	6.69***
Pulse pressure	32	48.0	-1.0	3.83	0.68	1.52	48.6	-1.6	4.50	0.80	2.04*
Heart rate	30	63.1	+11.7	8.56	1.56	7.47***	64.0	+9.3	6.29	1.15	8.12***
Stroke volume	30	111.0	-6.5	9.13	1.67	3.92***	115.2	-7.0	7.75	1.41	4.97***
Cardiac output	30	6.96	+0.78	0.89	0.16	4.82***	7.31	+0.55	0.81	0.15	3.60***

Tests IA, IB, IIA and IIB are further described in the text; this system of numbering is used throughout the tables for Series F.

*P < 0.05

**P < 0.01

***P < 0.001

Abbreviations: S.D. = standard deviation; S.E.M. = standard error of the mean.

From further evidence several factors on the influence of may be seen in the differences are compared. On the first day (I) control values are higher than on the second day. This is statistically significant. Lunch readings are the diastolic pressure.

Similar statistical analysis of one cigarette, are supplementary to those to smoking before pressure—is the change (IIA-IIB) systolic pressure rise before by many smokers any other, especially

TABLE III. MEAN D.

MEASUREMENT

Control value
Systolic pressure
Diastolic pressure
Pulse pressure
Heart rate
Stroke volume
Cardiac output
Change after smoking
Systolic pressure
Diastolic pressure
Pulse pressure
Heart rate
Stroke volume
Cardiac output

* P < 0.05

** P < 0.01

***P < 0.001

We have complementary, a ing) are quite ren the paired difference from zero which is

From further examination of Series F, it is possible to study the effect of several factors on the circulatory response to smoking.

The influence of time of day: The effect of time of day on the control readings may be seen in the upper part of Table III where the means of the paired differences are compared with their variances to give *t* values as tests of significance. On the first day (IA-IB), although all the differences are negative, i.e., the control values are higher before lunch than before breakfast, none of these differences is statistically significant. On the second day (IIA-IIB), however, all the pre-lunch readings are significantly higher than the prebreakfast readings excepting the diastolic pressure which is virtually the same in the two groups.

Similar statistical comparisons made of the change produced by smoking one cigarette, are set forth in the lower half of Table III. These results are complementary to those above. On the first day (IA-IB) there is a greater response to smoking before breakfast, although in only one measurement—the systolic pressure—is the change significant at the 5 per cent level. On the second day (IIA-IIB) systolic and pulse pressure, heart rate, and cardiac output show a bigger rise before breakfast. These findings tally with the subjective observation by many smokers that the first cigarette in the morning has more effect than any other, especially if it is smoked in the fasting state.

TABLE III. MEAN DIFFERENCES IN CONTROL VALUES AND IN CHANGES ON SMOKING (SERIES F)

MEASUREMENT	IA-IB		IIA-IIB		IA-IIA		IB-IIB	
	MEAN DIFFERENCE	<i>t</i>	MEAN DIFFERENCE	<i>t</i>	MEAN DIFFERENCE	<i>t</i>	MEAN DIFFERENCE	<i>t</i>
Control value								
Systolic pressure	-1.78	1.96	-2.59	2.91**	+0.31	0.21	-0.50	0.39
Diastolic pressure	-0.66	0.61	+0.03	0.05	-0.66	0.39	+0.03	0.02
Pulse pressure	-1.12	1.04	-2.62	2.92**	+0.97	0.58	-0.53	0.34
Heart rate	-2.23	1.97	-4.00	3.24**	+0.80	0.68	-0.97	0.69
Stroke volume	-1.57	0.72	-4.57	2.74*	-1.23	0.42	-4.23	2.11*
Cardiac output	-0.38	2.03	-0.71	4.39***	-0.03	0.14	-0.36	1.77
Change after smoking								
Systolic pressure	+2.94	2.48*	+2.19	2.52*	-1.16	0.88	-1.91	2.36*
Diastolic pressure	+1.59	1.40	-0.09	0.12	-0.81	0.67	-2.56	2.87**
Pulse pressure	+1.34	1.16	+2.28	2.36*	-0.34	0.29	+0.59	0.63
Heart rate	+1.67	1.17	+3.97	2.52*	+0.03	0.02	+2.33	1.66
Stroke volume	+0.37	0.16	+1.53	0.68	-0.67	0.27	+0.50	0.27
Cardiac output	+0.25	1.62	+0.54	3.14**	-0.07	0.35	+0.23	1.50

* *P* < 0.05** *P* < 0.01****P* < 0.001

We have commented above that the "control" and "change" figures are complementary, and, in fact, the "final" figures (or absolute readings after smoking) are quite remarkably constant (Table IV and Fig. 1). When "*t*" tests on the paired differences are examined on the null hypothesis, the only difference from zero which is significant is the stroke volume on the second day (Table V).

Tests IA, IB, IIA and IIB are further described in the text; this system of numbering is used throughout the tables for Series F.

P* < 0.05*P* < 0.01****P* < 0.001

Abbreviations: S.D. = standard deviation; S.E.M. = standard error of the mean.

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TABLE IV. COMPARISON OF MEANS OF CONTROL VALUES, CHANGES AND FINAL VALUES IN SERIES F SMOKING TESTS

MEASUREMENT	BEFORE BREAKFAST		BEFORE LUNCH	
	FIRST DAY	SECOND DAY	FIRST DAY	SECOND DAY
	IA	IIA	IB	IIB
Systolic pressure				
Control	113.2	112.9	115.0	115.5
Change	+ 5.4	+ 6.5	+ 2.4	+ 4.3
Final	118.6	119.4	117.4	119.8
Diastolic pressure				
Control	66.3	67.0	67.0	66.9
Change	+ 5.1	+ 5.9	+ 3.5	+ 6.0
Final	71.4	72.8	70.4	72.9
Pulse pressure				
Control	46.9	45.9	48.0	48.6
Change	+ 0.3	+ 0.7	- 1.0	- 1.6
Final	47.2	46.6	47.0	46.9
Heart rate				
Control	60.8	60.0	63.1	64.0
Change	+13.3	+13.3	+11.7	+ 9.3
Final	74.2	73.3	74.7	73.4
Stroke volume				
Control	109.4	110.6	111.0	115.2
Change	- 6.2	- 5.5	- 6.5	- 7.0
Final	103.2	105.1	104.4	108.2
Cardiac output				
Control	6.57	6.60	6.96	7.31
Change	+ 1.03	+ 1.10	+ 0.78	+ 0.55
Final	7.60	7.70	7.74	7.86

The adaptation factor: Since the IA test was the first smoking test performed on each of these subjects, it might be thought that on that occasion the subjects would be somewhat tense or apprehensive and that they would be more relaxed during subsequent tests. We have called the effects of familiarity with the test the "adaptation factor." This factor will be studied more closely and over a longer period in the analysis of Series E. In Series F, however, we can note what differences, if any, exist between the first and the second day. In the control values (Table III, IA-IIA, and IB-IIB) only the prelunch stroke volume is significantly different at the 5 per cent level. Comparison of the effect of smoking shows that the rises in systolic and diastolic pressures are significantly greater on the second day before lunch (Table III). None of the other differences is significant. Comparing the final figures shows no significant differences, although the trends are absolutely uniform: pulse pressures and heart rates are higher on the first than on the second day, while the other measurements are lower (Table V). It appears then that adaptation is an unimportant factor in the smoking test, a view which is confirmed in Series E.

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BEFORE LUNCH

ST DAY	SECOND DAY
IB	IIB
15.0 2.4 7.4	115.5 + 4.3 119.8
7.0 3.5 0.4	66.9 + 6.0 72.9
8.0 1.0 7.0	48.6 - 1.6 46.9
3.1 1.7 4.7	64.0 + 9.3 73.4
1.0 5.5 4.4	115.2 - 7.0 108.2
96 78 74	7.31 + 0.55 7.86

smoking test performed at this occasion the subjects would be more relaxed and familiar with the test procedure and over a longer period, however, we can note no significant differences on the second day. In the conclusion, stroke volume and the effect of smoking were significantly greater and the other differences in the differences, although heart rates are higher and measurements are lower and the most important factor in the smoking

MEAN RESPONSES TO SMOKING ON EACH OF FOUR OCCASIONS

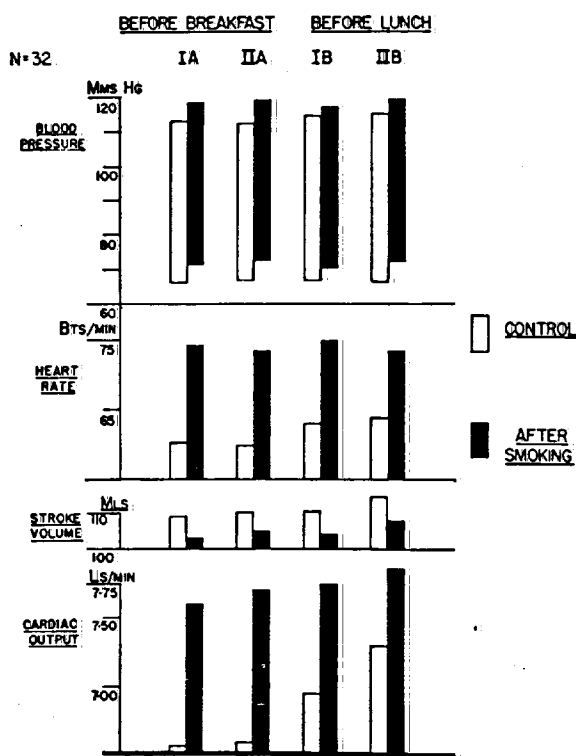


Fig. 1.—The relative constancy of the final postsmoking values under different circumstances. In order to represent small differences, only the upper portions of the columns for heart rate, stroke volume, and cardiac output are shown. For this reason, changes on smoking appear exaggerated.

classification, being all obviously very heavy or very light smokers. The division of subjects gave 12 lighter and 20 heavier smokers; as these numbers are small and any differences are also small, the results of all four tests were pooled for each smoking habit group, giving for the lighter smokers 48 complete sets of results and for the heavy smokers 80 sets of blood pressure readings and 72 sets of cardiac measurements (since both subjects with incomplete ballistocardiographic data were heavy smokers). The numbers of degrees of freedom in the comparisons between these two groups are to all intents and purposes infinitely large. Nothing was lost by pooling, since it transpired that the mean variance

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TABLE V. PAIRED DIFFERENCES BETWEEN FINAL VALUES IN FOUR SMOKING TESTS (SERIES F)

FINAL MEASUREMENTS	IA-IB		IIA-IBB		IA-IIA		IB-IIB		IA-IIB		IIA-IB	
	MEAN DIFFERENCE	t	MEAN DIFFERENCE	t	MEAN DIFFERENCE	t	MEAN DIFFERENCE	t	MEAN DIFFERENCE	t	MEAN DIFFERENCE	t
Systolic pressure	+1.16	0.87	-0.41	0.43	-0.84	0.48	-2.41	1.88	-1.25	0.68	+2.00	1.55
Diastolic pressure	+0.94	1.27	-0.06	0.07	-1.47	0.71	-2.47	1.41	-1.53	0.83	+2.41	1.24
Pulse pressure	+0.22	0.17	-0.34	0.34	+0.62	0.39	+0.06	0.04	+0.28	0.16	-0.41	0.27
Heart rate	-0.57	0.46	-0.03	0.02	+0.83	0.67	+1.37	0.88	+0.80	0.65	-1.40	0.71
Stroke volume	-1.20	0.79	-3.03	2.29*	-1.90	1.12	-3.73	1.92	-4.93	2.36*	+0.70	0.41
Cardiac output	-0.13	0.87	-0.17	0.95	-0.09	0.05	-0.13	0.71	-0.26	1.41	-0.04	0.17

* P < 0.05.

in the pooled group of individual groups of more than half as great as the standard errors (Table VI). The relationship between the values and the

TABLE VI. THE I

MEASUREMENTS

Systolic pressure
Control
Change
Final

Diastolic pressure
Control
Change
Final

Pulse pressure
Control
Change
Final

Heart rate
Control
Change
Final

Stroke volume
Control
Change
Final

Cardiac output
Control
Change
Final

Where in the values are calculated

* P < 0.05.

** P < 0.01.

much more evident level. There are the mean values of IIB separately are laid out in IB, IIA, and a smaller heart rate was

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in the pooled group in each instance was very little more than that for the individual group of tests. It is clear, then, that the standard error was little more than half as great (since the number of observations is four times as great and the standard error varies inversely as the square root of the number of observations). The results obtained, set forth in Table VI, do not show much difference between the two groups. Lighter smokers have almost always higher control values and show less change on smoking. The results in the final figures are

TABLE VI. THE EFFECTS OF SMOKING HABITS ON THE RESULTS OF THE SMOKING TESTS (SERIES F)

MEASUREMENT	LIGHT SMOKERS (L) MEAN	HEAVY SMOKERS (H) MEAN	L-H	t
Systolic pressure				
Control	116.65	112.66	+ 3.99	2.910**
Change	+ 3.44	+ 5.41	- 1.97	1.910
Final	120.08	118.08	+ 2.01	1.079
Diastolic pressure				
Control	67.79	66.20	+ 1.59	1.144
Change	+ 4.96	5.18	- 0.22	0.227
Final	72.75	71.38	+ 1.37	0.723
Pulse pressure				
Control	48.85	46.46	+ 2.39	1.737
Change	- 1.52	+ 0.24	- 1.76	2.350*
Final	47.33	46.70	+ 0.63	0.418
Heart rate				
Control	63.92	60.71	+ 3.21	2.088*
Change	+ 9.98	+13.19	- 3.21	1.720
Final	73.90	73.90	0.00	0.000
Stroke volume				
Control	110.33	112.36	- 2.03	0.689
Change	- 5.81	- 6.64	+ 0.83	0.493
Final	104.52	105.72	- 1.20	0.451
Cardiac output				
Control	7.03	6.75	+ 0.28	1.315
Change	+ 0.65	+ 1.01	- 0.36	1.863
Final	7.68	7.76	- 0.08	0.323

Where in the above comparisons variance is significantly different in the two groups, probability values are calculated from weighted t values (Cochran's technique).

* P < 0.05.

**P < 0.01.

much more evenly distributed and none of the differences reaches the significant level. There are no grounds for pairing the individual subjects, but if we compare the mean values for lighter and heavier smokers in tests IA, IB, IIA, and IIB separately, we can study consistency of trends. The results so obtained are laid out in Table VII. This shows, for example, that in all four tests (IA, IB, IIA, and IIB) the lighter smokers had a higher average control heart rate and a smaller average rise in heart rate on smoking, but that the average final heart rate was higher in two instances and lower in the other two.

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TABLE VII. CONSISTENCY OF TRENDS OF MEAN VALUES IN THE FOUR GROUPS OF TESTS COMPARING THE LIGHTER AND HEAVIER SMOKERS (SERIES F)

MEASUREMENT	CONTROL		CHANGE		FINAL	
	LIGHTER SMOKERS	HEAVIER SMOKERS	LIGHTER SMOKERS	HEAVIER SMOKERS	LIGHTER SMOKERS	HEAVIER SMOKERS
Systolic pressure	4	0	1	3	3	1
Diastolic pressure	3	1	1	3	2	2
Pulse pressure	3	1	0	4	2	2
Heart rate	4	0	0	4	2	2
Stroke volume	1	3	3	1	1	3
Cardiac output	4	0	0	4	1	3

The numbers in Tables VII indicate in how many of the four groups of comparisons the mean value is the higher (in the direction of positivity) in the group concerned.

From the four such comparisons that can be made, it is possible to obtain only an over-all impression: that in the main the control values are higher more frequently in the lighter smokers, the change greater in the heavier smokers, and the final readings evenly distributed.

Effect of exercise habits: Since it is difficult to quantitate every-day exercise accurately, in studying its effect upon the response to smoking we have made a broad division as in the preceding section. Inquiry into the amount and kind of exercise during the preceding month was made at the time of each smoking test and the subjects classified as taking "no," "little," "moderate," or "much" exercise according to prearranged definitions. Two main groups were then formed by combining those taking no and little exercise on the one hand and moderate and much on the other. The resulting groups were of equal size. As before, the A and B tests were pooled with the same advantage from the reduction in size of the standard errors with little ill effect from slight loss of homogeneity of the material. The resulting groups are compared by mean values (Table VIII). The differences are not great: the less active subjects have a higher resting heart rate with a bigger rise on smoking and this is reflected in a greater rise and a higher final figure for the cardiac output. Other differences are not statistically significant.

It might be thought that the factors of smoking habits and exercise might influence each other: that the more athletic students are more sociable and more likely to smoke heavily; or conversely, that many of them are likely to be light smokers as a result of athletic training. In this way one would be comparing two groups, the composition of which differs in more than one respect. Accordingly, the association between these two factors has been analyzed (Table IX). It is clear that even if we look upon all the tests as having been performed on different individuals instead of four of them by each, the value for chi-square is not significant at the 5 per cent level, though it does seem that the less athletic subjects smoke more.

TABLE VIII. THE

MEASUREMENT

Systolic pressure
Control
Change
Final

Diastolic pressure
Control
Change
Final

Pulse pressure
Control
Change
Final

Heart rate
Control
Change
Final

Stroke volume
Control
Change
Final

Cardiac output
Control
Change
Final

Where in the
values are calculated

* $P < 0.05$.

** $P < 0.01$.

TABLE IX.

EXERCISE HABITS
Much Little
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GROUPS OF TESTS COMPARING
S F)

R S	FINAL	
	LIGHTER SMOKERS	HEAVIER SMOKERS
	3	1
	2	2
	2	2
	2	2
	1	3
	1	3

of comparisons the mean value

it is possible to obtain
values are higher more
in the heavier smokers,

itate every-day exercise
noking we have made a
o the amount and kind
e time of each smoking
"moderate," or "much"
groups were then formed
one hand and moderate
equal size. As before,
e from the reduction in
ht loss of homogeneity
an values (Table VIII).
s have a higher resting
flected in a greater rise
ifferences are not statis-

bits and exercise might
more sociable and more
m are likely to be light
e would be comparing
one respect. Accord-
analyzed (Table IX).
ing been performed on
value for chi-square is
that the less athletic

TABLE VIII. THE EFFECTS OF EXERCISE HABITS ON THE RESULTS OF THE SMOKING TEST (SERIES F)

MEASUREMENT	LIGHT EXERCISE (L) MEAN	HEAVY EXERCISE (H) MEAN	L-H	t
Systolic pressure				
Control	113.48	114.83	-1.34	1.003
Change	4.06	5.28	-1.22	1.215
Final	117.55	120.11	-2.56	1.623
Diastolic pressure				
Control	65.58	68.02	-2.44	1.887
Change	4.64	5.55	-0.91	0.997
Final	70.22	73.56	-3.34	1.917
Pulse pressure				
Control	47.91	46.81	1.09	0.816
Change	-0.58	-0.27	-0.31	0.404
Final	47.33	46.55	0.78	0.542
Heart rate				
Control	63.42	60.36	3.06	2.038*
Change	13.47	10.12	3.34	1.940
Final	76.89	70.48	6.41	3.093**
Stroke volume				
Control	109.55	113.84	-4.29	1.502
Change	-4.97	-7.84	2.87	1.780
Final	104.58	106.00	-1.42	0.545
Cardiac output				
Control	6.89	6.82	0.07	0.358
Change	1.11	0.59	0.52	2.927**
Final	8.00	7.41	0.59	2.633*

Where in the above comparisons variance is significantly different in the two groups, probability values are calculated from weighted t values (Cochran's technique).

* P < 0.05.

**P < 0.01.

TABLE IX. CORRELATION BETWEEN THE EXERCISE AND SMOKING HABITS OF THE SUBJECTS

EXERCISE HABITS	SMOKING HABITS				TOTAL
	HEAVY SMOKERS		LIGHT SMOKERS		
	OBS.	EXP.	OBS.	EXP.	
Much	36	40	28	24	64
Little	44	40	20	24	64
Total	80		48		128

$$\chi^2 = 1.633 \text{ } p < 0.2$$

The constancy of ceiling: One interesting fact that emerged from this analysis is that the final figure immediately after smoking was the most constant reading (Table IV, Fig. 1). These "ceiling" values varied so little that even if

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TABLE X. VARIABILITY OF INDIVIDUAL RESPONSE TO SMOKING: MEAN VALUES AND STANDARD DEVIATIONS OF CIRCULATORY MEASUREMENTS IN EIGHT TESTS ON EACH OF SIX SUBJECTS (SERIES E)

SUBJECT NO.	VALUE	SYSTOLIC PRESSURE		DIASTOLIC PRESSURE		PULSE PRESSURE		HEART RATE		STROKE VOLUME		CARDIAC OUTPUT	
		MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
58127	Control	125.8	3.2	65.8	6.2	60.0	6.0	52.1	3.6	110.8	7.7	5.8	0.5
	Change	+12.5**	7.0	+12.2***	3.0	+ 0.2	6.9	+17.2***	5.3	- 4.3	12.2	+1.6**	1.0
	Final	138.3	7.9	78.0	4.4	60.2	6.3	69.3	2.8	106.5	8.6	7.4	0.7
58158	Control	110.9	3.6	65.6	3.0	45.2	4.7	69.8	7.7	99.7	8.9	6.9	0.7
	Change	+ 2.9	3.6	+ 7.0***	1.7	- 4.1*	4.8	+19.1***	4.9	- 9.5**	7.3	+1.1***	0.4
	Final	113.8	3.1	72.6	2.6	41.1	3.6	88.9	5.7	90.2	4.6	8.0	0.6
59170	Control	94.4	3.7	61.0	1.7	33.4	3.1	66.9	4.7	120.1	6.5	8.0	0.7
	Change	0.0	2.0	+ 0.8	2.4	- 0.8	1.7	+ 5.5**	4.4	+ 2.3	10.7	+0.8**	0.5
	Final	94.4	3.9	61.8	3.0	32.6	2.9	72.4	7.8	122.4	11.4	8.8	0.7
58102	Control	145.0	3.8	79.1	4.4	65.9	4.7	65.9	4.7	134.4	9.9	8.8	0.6
	Change	+ 3.5	5.9	+ 4.6	5.4	- 1.1	5.1	+10.4***	5.2	- 6.6*	8.5	+0.9**	0.5
	Final	148.5	4.9	83.7	3.7	64.8	2.9	76.3	6.3	127.8	7.7	9.7	0.4
59118	Control	112.1	5.9	70.8	8.1	41.4	4.6	61.1	7.5	124.5	6.7	7.6	0.8
	Change	+ 0.9	6.0	+ 1.0	2.7	- 1.9	4.0	+ 1.8	3.0	- 3.2	7.4	0.0	0.6
	Final	111.2	5.0	71.8	8.8	39.5	5.6	63.0	6.4	121.3	5.6	7.6	0.8
58152	Control	116.8	5.4	71.5	4.0	45.2	5.3	72.7	4.6	99.2	3.6	7.2	0.4
	Change	+ 0.9	2.5	+ 3.4	5.5	- 2.5	5.7	+ 1.2	5.5	+ 0.5	7.1	+0.2	0.4
	Final	117.6	4.3	74.9	5.1	42.7	3.8	73.9	5.6	99.7	5.1	7.4	0.5
Mean	Control	117.5	4.4	69.0	5.1	48.5	4.8	64.8	5.7	114.8	7.5	7.4	0.6
	Change	+ 3.1	4.9	+ 4.8	3.8	- 1.7	5.0	+ 9.2	4.8	- 3.5	8.1	+0.8	0.6
	Final	120.6	5.1	73.8	5.0	46.8	4.4	74.0	6.0	111.3	7.5	8.2	0.7

The probability estimates, showing that the change on smoking is significant, are made from "t" tests which compare the mean change on smoking with the variability of the mean change on smoking.

S.D. = standard deviation.

*P < 0.05.

**P < 0.01.

***P < 0.001.

the readings obtained before breakfast on the 36 combination level—about the first reservation suggests that regardless of the effect for lighter and heavier affect it.

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PERCENTAGE CHANGE AFTER ONE CIGARETTE

Source: <https://www.industrydocuments.ucsf.edu/docs/gfml0000>

Fig. 2.—True individual changes in eight tests on each of six subjects (Series E) and Fig. 3.

The reproducibility of the readings on subjects under varying degrees of varying degrees (Series E) shows the average for each function: root mean variance. The result characteristics of varying degrees (Series E), were ref

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the readings obtained under the most unlike circumstances are paired (i.e., those before breakfast on one day with those before lunch on the other) only two of the 36 combinations (5.6 per cent) are significantly different at the 5 per cent level—about the frequency one would expect by chance (Table V). This observation suggests that smoking a cigarette tends to push the various measurements to a ceiling, as if a cigarette at different times of day "takes up the slack" regardless of the control figure. Moreover, the ceiling was essentially the same for lighter and heavier smokers, and differences in habits of exercise did not affect it.

MEAN PATTERNS OF EIGHT SMOKING TESTS
FOR EACH OF SIX SUBJECTS

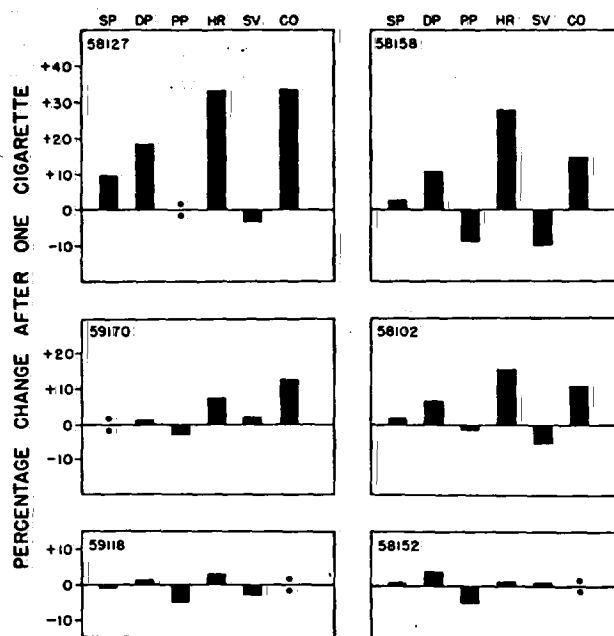


Fig. 2.—The individual circulatory response to smoking. Each column represents the mean of the changes in eight tests. The subjects are arranged in order of reactivity. The same order is used in Table X and Fig. 3.

The reproducibility and individuality of the measurements on repeated tests on subjects under standardized conditions: the series of eight tests on each of 6 subjects (Series E).—The results obtained are summarized in Table X which shows the average and standard deviation for each function measured in the six subjects on eight occasions. The bottom row shows the means of the 48 readings for each function with a mean of individual standard deviation (calculated as root mean variance). Fig. 2 shows the mean response to smoking of each subject. The results of repeated tests indicate that: (1) on the average, individual characteristics were maintained throughout the series of tests; (2) persons of varying degrees of reactivity, from marked (Subject 58127) to slight (Subject 58152), were represented among the test subjects. As might be expected, when

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The probability estimates, showing that the change on smoking is significant, are made from "t" tests which compare the mean change on smoking with the variability of the mean change on smoking.

S.D. = standard deviation.

*P < 0.05.

**P < 0.01.

***P < 0.001.

Mean	Final	Initial	4.5	4.9	5.1	42.1	13.9	5.0	99.7	5.1	7.4	0.5
Control	117.5	117.0	4.4	69.0	5.1	48.5	4.8	5.7	114.8	7.5	7.4	0.6
Change	+3.1	4.9	4.9	+4.8	3.8	-1.7	5.0	4.8	-3.5	8.1	+0.8	0.6
Final	120.6	5.1	73.8	5.0	46.8	4.4	74.0	6.0	111.3	7.5	8.2	0.7

the subjects are arranged in order of decreasing responsiveness, most of the mean changes which are significantly greater than the standard deviations appear in the upper portion of the table (Table X).

From the average standard deviations, we have attempted to estimate the reliability of a single test on one subject, by taking twice this value as the 95 per cent confidence limits (Table XI). Thus the control reading for the systolic pressure, for example, in one test on a given subject will lie within 8.8 mm. Hg either way of the population mean for that person, provided that: (1) one such test is an unbiased estimate of the mean; and (2) differences between the variances of the several individuals are entirely explicable by chance and do not denote that some subjects are more variable in their response than others.

TABLE XI. CONFIDENCE LIMITS ON THE RESULTS OF A SINGLE TEST (SERIES E)

MEASUREMENT	MEAN STANDARD DEVIATION (ROOT MEAN VARIANCE)	MEAN MEAN	95% CONFIDENCE LIMITS (ABSOLUTE)
Systolic pressure			
Control	4.4	117.5	± 8.8
Change	4.9	+ 3.1	± 9.8
Final	5.1	120.6	± 10.2
Diastolic pressure			
Control	5.1	69.0	± 10.2
Change	3.8	+ 4.8	± 7.6
Final	5.0	73.8	± 10.0
Pulse pressure			
Control	4.8	48.5	± 9.6
Change	5.0	- 1.7	± 10.0
Final	4.4	46.8	± 8.8
Heart rate			
Control	5.7	64.8	± 11.4
Change	4.8	+ 9.2	± 9.6
Final	6.0	74.0	± 12.0
Stroke volume			
Control	7.5	114.9	± 15.0
Change	9.1	- 3.6	± 18.2
Final	7.5	111.3	± 15.0
Cardiac output			
Control	0.6	7.4	± 1.2
Change	0.6	+ 0.8	± 1.2
Final	0.7	8.2	± 1.4

That the first premise is a valid assumption is seen in Table XII, where the results of the set of six first tests are averaged, then the second tests, the third, and so on. The averages of the first tests differ very little from the averages of the subsequent tests and are a very good estimate of the mean for all 48 tests. Similar results are obtained if the results are ranked. The second premise has been examined by Bartlett's test for homogeneity of variance, the results being presented in Table XIII. In 13 of the readings, differences appear to be no more than could be readily explained by chance. In the other five, including all three

TABLE XII. COMP

MEASUREMENT	FI
Systolic pressure	
Control	11
S.D.	1
Change	
S.D.	
Final	12
S.D.	1
Diastolic pressure	
Control	7
S.D.	
Change	
S.D.	
Final	
S.D.	
Pulse pressure	
Control	
S.D.	
Change	
S.D.	
Final	
S.D.	
Heart rate	
Control	
S.D.	
Change	
S.D.	
Final	
S.D.	
Stroke volume	
Control	
S.D.	
Change	
S.D.	
Final	
S.D.	
Cardiac output	
Control	
S.D.	
Change	
S.D.	
Final	
S.D.	

S.D. = Standard

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TABLE XII. COMPARISON OF THE MEAN VALUES OF EIGHT SETS OF TESTS (SERIES E)

MEASUREMENT	FIRST	SECOND	THIRD	FOURTH	FIFTH	SIXTH	SEVENTH	EIGHTH	ALL
Systolic pressure									
Control	119.7	115.3	116.2	118.5	119.5	119.2	114.2	117.3	117.5
S.D.	18.1	14.3	15.1	18.6	17.0	16.7	19.6	19.0	16.2
Change	1.0	6.0	3.2	3.0	2.5	3.7	4.2	1.7	3.1
S.D.	4.0	2.3	5.7	8.5	9.4	9.7	7.5	2.7	6.5
Final	120.7	121.3	119.3	121.5	122.0	122.8	118.3	119.0	120.6
S.D.	17.8	15.5	20.5	22.8	20.6	22.9	21.0	19.4	18.7
Diastolic pressure									
Control	70.2	67.8	67.2	68.8	69.2	70.8	68.8	68.8	69.0
S.D.	8.8	6.3	7.7	7.1	6.5	7.3	11.8	7.4	7.5
Change	4.3	6.3	7.5	5.2	4.3	5.2	2.0	3.8	4.8
S.D.	6.2	5.8	4.5	6.7	5.3	5.4	5.6	3.2	5.3
Final	74.5	74.2	74.7	74.0	73.5	76.0	70.8	72.7	73.8
S.D.	8.4	5.3	10.5	7.3	7.6	8.3	12.5	8.9	8.3
Pulse pressure									
Control	49.5	47.5	49.0	49.7	50.3	48.3	45.3	48.5	48.5
S.D.	16.4	14.0	11.0	15.3	11.3	11.8	10.4	12.4	12.1
Change	-3.3	-0.3	-4.3	-2.2	-1.8	-1.5	2.2	-2.2	-1.7
S.D.	5.1	4.5	5.5	5.2	6.5	5.2	3.8	2.5	4.9
Final	46.2	47.2	44.7	47.5	48.5	46.8	47.5	46.3	46.8
S.D.	12.4	11.1	12.0	16.8	14.6	16.2	12.1	10.8	12.5
Heart rate									
Control	63.3	66.8	64.5	64.6	69.0	63.4	60.5	66.1	64.8
S.D.	10.4	6.1	9.1	8.3	11.9	8.8	10.3	5.1	8.6
Change	8.7	9.2	7.6	11.1	8.0	9.6	10.4	9.2	9.2
S.D.	8.5	8.8	12.0	6.1	7.5	10.8	9.4	7.8	8.4
Final	72.0	76.0	72.1	75.7	77.0	73.0	70.9	75.2	74.0
S.D.	14.4	9.5	11.6	9.5	9.6	8.0	10.8	7.7	9.8
Stroke volume									
Control	116.7	116.2	114.1	113.0	115.4	116.1	114.3	112.4	114.8
S.D.	17.1	15.8	19.3	15.7	22.6	13.3	8.2	11.2	14.8
Change	-0.5	-2.6	-2.5	-2.3	-4.4	-8.0	-5.8	-1.7	-3.5
S.D.	12.4	10.9	9.9	11.3	8.7	9.0	10.7	5.0	9.5
Final	116.2	113.6	111.6	110.6	111.0	108.1	108.4	110.7	111.3
S.D.	19.5	15.4	20.1	17.5	18.4	13.2	15.7	9.4	15.4
Cardiac output									
Control	7.34	7.77	7.32	7.27	7.60	7.35	6.90	7.42	7.40
S.D.	1.30	1.24	1.24	1.18	1.15	1.28	1.18	0.87	1.13
Change	0.83	0.83	0.57	0.99	0.64	0.54	0.73	0.90	0.75
S.D.	0.75	0.64	1.18	1.26	0.61	0.55	0.72	0.73	0.79
Final	8.18	8.60	7.90	8.26	8.44	7.89	7.63	8.32	8.15
S.D.	1.08	1.29	0.88	0.71	1.07	1.27	1.31	1.04	1.06

S.D. = Standard deviation.

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diastolic readings, individual subjects seem to differ widely. Moreover, our main concern is with the change on smoking, and it transpires that the variability of the *change* of all three blood pressure readings, systolic, diastolic, and pulse pressure, differs significantly from individual to individual.

TABLE XIII. BARTLETT'S TEST FOR HOMOGENEITY OF VARIANCE IN THE DATA FROM SERIES E

MEASUREMENT	M'	p
Systolic pressure		
Control	4.206	0.75
Change	14.557*	0.025
Final	7.206	0.25
Diastolic pressure		
Control	16.885**	0.005
Change	13.99*	0.025
Final	14.107*	0.025
Pulse pressure		
Control	2.857	0.75
Change	11.263*	0.05
Final	7.524	0.25
Heart rate		
Control	6.072	0.5
Change	2.777	0.75
Final	6.211	0.5
Stroke volume		
Control	6.821	0.25
Change	3.958	0.75
Final	8.227	0.25
Cardiac output		
Control	3.499	0.75
Change	8.399	0.25
Final	3.912	0.75

M' = Bartlett's index for homogeneity of variance (M), corrected for small sample size. A significant value for M' indicates that variance differs from one subject to another. It is read as a χ^2 value (degrees of freedom: 6-1 = 5).

* P < 0.05.

**P < 0.01.

It seems then that our confidence limits may be applied to the first smoking test; and, in an individual test, probably to the heart rate, stroke volume, and cardiac output, but not to the blood pressure values. In other words, not only the mean, but also the variance is an individual characteristic: for some subjects, our confidence limits are too broad, and for others, too narrow. We have no way of predicting which of these errors is likely in any particular subject; regressions of variance on the mean show no significant correlation. In the main, of course, we will be able to distinguish "hypo-" from "hyper-" reactors, but from time to time we may make errors, and it is perhaps best to apply our confidence limits, especially for the blood pressure responses, to groups only. A standard error is easily computed from our confidence limits.

The object of the smoking test is to characterize a given subject according to the degree and direction of change in regard to each variable, and the success

of the test depends on repeated test; the test is reliable; the interindividual and interindividual procedure. Th

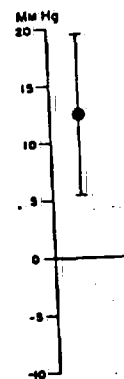


Fig. 3.—Inter-individual variance: the black circle represents the mean; the vertical line represents the range with which subjects

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ely. Moreover, our
es that the variability
c, diastolic, and pulse
l.

THE DATA FROM SERIES E

p
0.75
0.025
0.25
0.005
0.025
0.025
0.75
0.05
0.25
0.5
0.75
0.5
0.25
0.75
0.25
0.75
0.75

all sample size. A signifi-
It is read as a χ^2 value

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of the test depends on the contrast between individual variation (intraindividual) on repeated tests and variation between individuals (interindividual). A single test is reliable if the intraindividual scatter is relatively small compared with the interindividual variation, but if there is not much difference between the intra- and interindividual variation, then the test will be of little value as a screening procedure. This question is best answered by an analysis of variance (Fig. 3).

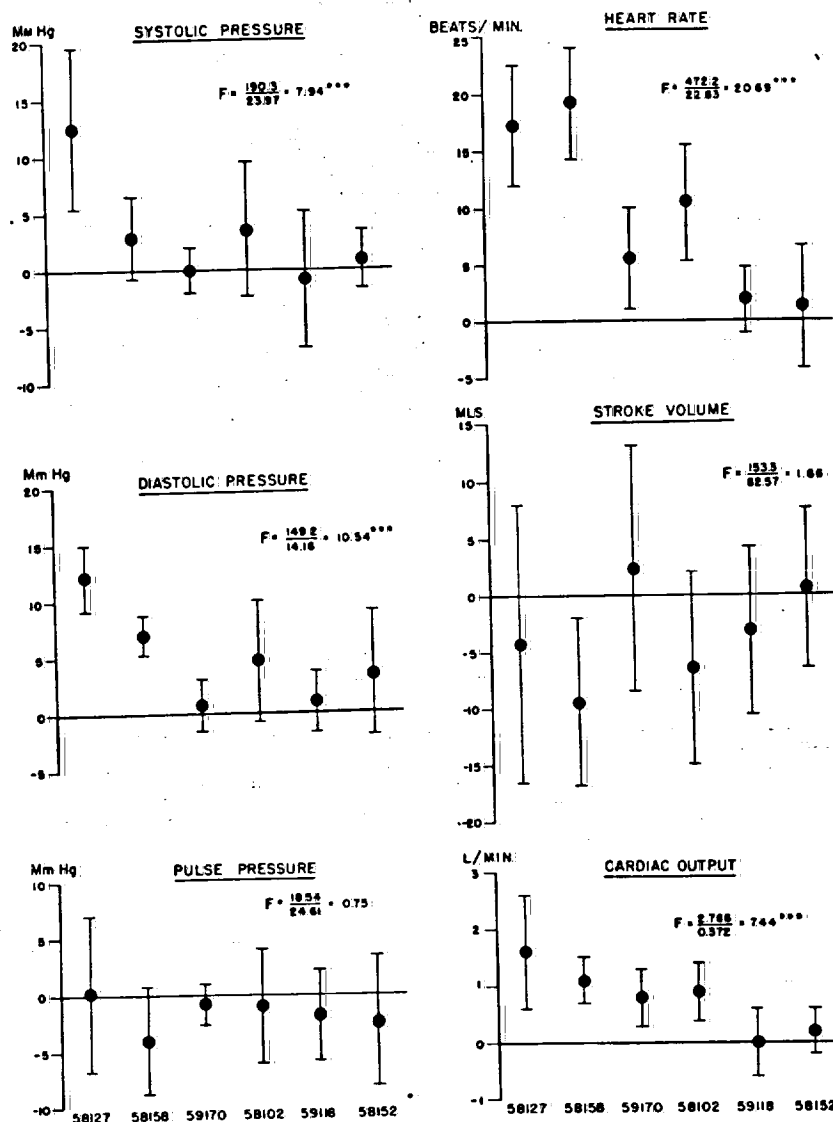


Fig. 3.—Intraindividual variation in eight smoking tests on each of 6 subjects. In each instance, the black circle marks the mean value and the lines one standard deviation above and below it. F represents the ratio of interindividual to intraindividual variance and is an indication of the confidence with which subjects may be classified according to the magnitude of the response. ***P < 0.001.

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Since we are chiefly concerned with *change* on smoking, the data concerning individuality of resting values, which is a matter of common experience, will not be presented here.

The variance obtained by pooling all 48 sets of readings of the change in systolic pressure after smoking, for example, arises from two sources: intraindividual variation as shown in Table X and interindividual variation which is found by subtracting the intraindividual variation from the total variation. We can then set up an analysis of variance as follows:

Source of variation	Degrees of freedom	Sum of squares	Mean square
Interindividual	$6 - 1 = 5$	951	190.3
Intraindividual	$6(8 - 1) = 42$	1007	23.97
Total	$(6 \times 8) - 1 = 47$	1958	—

The ratio of the two mean squares (interindividual variation being always the numerator) is designated "F" and corresponding values for p are obtained from appropriate tables with (5,42) degrees of freedom. The value for F in this example is 7.94 which gives $p < 0.001$. From analyses similar to this we find that changes in systolic and diastolic pressure, heart rate, and cardiac output are fairly individual, and are likely to produce a satisfactory subdivision of our subjects, whereas changes in pulse pressure and stroke volume are not, except perhaps in rare instances. These findings agree with our previous general impressions.

TABLE XIV. COMPARISON OF VARIANCE IN THE CONTROL AND FINAL FIGURES IN SERIES E

SUBJECT NO.	VALUE	SYSTOLIC PRESSURE	DIASTOLIC PRESSURE	PULSE PRESSURE	HEART RATE	STROKE VOLUME	CARDIAC OUTPUT
58127	Control	10.00	38.86	36.57	13.33	58.60	0.284
	Final	62.29	19.71	40.29	7.95	74.74	0.519
	r	-0.77*	+0.61	-0.05	+0.27	-0.12	-0.30
58158	Control	13.29	9.12	21.93	59.30	80.04	0.454
	Final	9.36	6.86	12.70	31.98	21.03	0.398
	r	+0.19	+0.25	+0.28	+0.44	+0.66	+0.10
59170	Control	13.43	2.86	9.71	22.14	41.89	0.490
	Final	15.14	8.86	8.57	51.52	128.86	0.507
	r	-0.12	-0.59	+0.12	-0.73*	-0.54	-0.03
58102	Control	14.57	19.27	22.13	21.74	98.12	0.353
	Final	24.00	13.36	8.50	39.06	58.64	0.176
	r	-0.25	+0.18	+0.45	-0.34	+0.30	+0.38
59118	Control	34.70	65.64	21.13	55.81	44.68	0.628
	Final	25.36	77.93	31.71	41.43	30.88	0.714
	r	+0.17	-0.27	-0.28	+0.35	+0.19	-0.11
58152	Control	28.79	16.00	28.64	21.13	12.83	0.160
	Final	18.27	26.14	14.21	31.29	26.21	0.267
	r	+0.44	-0.25	+0.33	-0.21	-0.36	-0.31

*P < 0.05. r = correlation coefficient.

It is of some interest to note the readings in Series E. in Table XIV. The two readings is by Pitances of paired measurements obtained are significant be expected to occur in E are any less variable was found in Series I test conditions of Series were made at a single obtained before breathing conditions.

Comparison between Tests.—We have the rigorously standardized influence the size of the with the different degrees fact these materially

In Table XV the instance the variance of the F value so obviously significantly greater Series while if significant of the 72 values obtained 1 per cent level. No recognizable pattern greater than one (6 ability) and in 9 significant 1 per cent level). and all of the 1 per seen to occur in the heart rate, stroke volume are of more importance cases it is necessary two groups of unequal XVI we have compared single tests on the other. The significance is no very striking difference, in most instances on smoking higher

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It is of some interest to compare the variability of the control and the final readings in Series E. The variances for each kind of measurement are set forth in Table XIV. The most sensitive way of detecting differences between these two readings is by Pitman's technique, which is designed for comparing the variances of paired measurements.³ Only two of the 36 correlation coefficients so obtained are significant at the 5 per cent level, and since this is about what would be expected to occur by chance, there is no evidence that final readings in Series E are any less variable than the control readings. This marked contrast to what was found in Series F remains to be explained but may will lie in the different test conditions of Series E and Series F: in Series E, all eight control readings were made at a single time of day, whereas in Series F, two control readings were obtained before breakfast and two before lunch under somewhat different conditions.

Comparison between the Results Obtained in the Different Series of Smoking Tests.—We have thus far considered the reproducibility of the results under rigorously standardized conditions and also some of the factors which seem to influence the size of the response. It now remains to compare the results obtained with the different degrees of standardization of the test conditions to seen if in fact these materially affect the results.

In Table XV the variances in Series O and Series F are compared. In each instance the variance in the former is divided by that in the latter, the significance of the F value so obtained being read from two-tailed tables. If the values are significantly greater than one then the variance is significantly greater in the O Series while if significantly less than one the converse is true. It is apparent that of the 72 values obtained, 19 are significantly different from one, 8 of them at the 1 per cent level. Notwithstanding these marked differences, there is no readily recognizable pattern in the results: in 10 instances, the variance is significantly greater than one (6 at the 5 per cent level and 4 at the 1 per cent level of probability) and in 9 significantly less than one (5 at the 5 per cent level and 4 at the 1 per cent level). There appears to be a definite trend: most of the 5 per cent and all of the 1 per cent probabilities in which the Series O reading is greater are seen to occur in the blood pressure readings, and the converse appears for the heart rate, stroke volume, and cardiac output. These comparisons of variance are of more importance in comparing the mean values, since in the appropriate cases it is necessary to calculate "t" values by Cochran's technique, for comparing two groups of unequal numbers and significantly different variances. In Table XVI we have computed the algebraic differences between the means of the 69 single tests on the one hand and those of the four sets of tests in Series F on the other. The significance of these differences is indicated in the usual way. There is no very striking pattern, but it does appear that where there is a significant difference, in most instances, the control readings are higher in Series O, the change on smoking higher in Series F, and the final readings about evenly distributed.

Series O is compared with the eight tests in Series E in Tables XVII and XVIII. The former compares the variances by the same method as in Table XV. Twenty-one of the 144 results are significantly different from unity: 14

AL FIGURES IN SERIES E

STROKE VOLUME	CARDIAC OUTPUT
58.60 74.74 -0.12	0.284 0.519 -0.30
80.04 21.03 +0.66	0.454 0.398 +0.10
41.89 128.86 -0.54	0.490 0.507 -0.03
98.12 58.64 +0.30	0.353 0.176 +0.38
44.68 30.88 +0.19	0.628 0.714 -0.11
12.83 26.21 -0.36	0.160 0.267 -0.31

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TABLE XV. COMPARISON OF THE VARIANCE IN SERIES O AND F

MEASUREMENT	VARIANCE RATIOS			
	O	O	O	O
	IA	IIA	IB	IIB
Systolic pressure:				
Control	2.44**	1.30	1.65	1.18
Change	0.855	0.870	3.70**	1.33
Final	1.35	0.926	2.32*	1.01
Diastolic pressure				
Control	0.735	0.833	0.855	1.23
Change	0.800	0.763	1.37	0.952
Final	0.549*	0.485*	0.714	0.658
Pulse pressure				
Control	1.44	1.62	1.91*	1.34
Change	2.19*	2.47**	3.68**	2.23*
Final	1.87	1.88	1.51	1.11
Heart rate:				
Control	1.60	1.88	2.03*	1.81
Change	0.452**	0.330**	0.654	1.21
Final	1.08	0.671	1.29	0.935
Stroke volume				
Control	1.01	1.08	0.990	1.09
Change	0.451**	0.490*	0.526*	0.730
Final	1.44	2.09*	1.40	1.33
Cardiac output				
Control	1.88	1.64	1.31	1.44
Change	0.543*	0.351**	0.714	0.847
Final	1.60	1.10	1.50	1.20

*P < 0.05. **P < 0.01.

of these (6 at the 5 per cent level and all 8 at the 1 per cent level) occurred in the systolic pressure readings, only 5 occurring in the remaining 120 values and all at the 5 per cent level. In almost all instances these values are significantly less than unity, a reflection of the fact that the groups of subjects being compared are of such unequal sizes (69 and 6, respectively) which means that the variance in group O has to be relatively very much larger to reach a significant level. Thus if we examine all readings other than systolic pressure, we find that 64 values are less than unity and 56 more. Again, however, in the systolic readings, only 4 of the 24 values are greater than unity. It seems, then, as if the only real difference in the two groups is that the systolic pressure is almost uniformly more variable between individuals in conditions standardized for each individual separately (but not uniform from one individual to another) than that within a large group of individuals under random conditions. The significance of this is obscure unless it be that there was a different observer in Series E (E.A.M.) or the fact that the latter subjects were in part selected. As before, we are mainly interested in using variance to assess the significance of differences in the mean values. It is clear from Table XVII that these are unimpressive.

TABLE XVI.

MEASUREMENT
Control
Systolic pressure
Diastolic pressure
Pulse pressure
Heart rate
Stroke volume
Cardiac output
Change
Systolic pressure
Diastolic pressure
Pulse pressure
Heart rate
Stroke volume
Cardiac output
Final
Systolic pressure
Diastolic pressure
Pulse pressure
Heart rate
Stroke volume
Cardiac output

* P < 0.05.
** P < 0.01.
***P < 0.001.

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TABLE XVI. COMPARISON BETWEEN THE MEAN VALUES IN SERIES O AND F

MEASUREMENT	O-IA	O-IIA	O-IB	O-IIB	SIGNIFICANT DIFFERENCES	
					POSITIVE	NEGATIVE
Control						
Systolic pressure	1.0	1.3	-0.8	-1.3	0	0
Diastolic pressure	7.9***	4.6**	4.6**	4.7***	4	0
Pulse pressure	-4.3*	-3.3	-5.4**	-6.0***	0	3
Heart rate	10.0***	10.8***	7.7***	6.8**	4	0
Stroke volume	0.3	-0.9	-1.3	-10.5**	0	1
Cardiac output	1.12***	1.09***	0.73**	0.38	3	0
Change						
Systolic pressure	-2.8*	-3.9**	0.2	-1.7	0	2
Diastolic pressure	-0.2	-1.0	1.2	-1.1	0	0
Pulse pressure	-2.6*	-3.0**	-1.3	-0.7	0	2
Heart rate	-5.8**	-5.8*	-4.2*	-1.8	0	3
Stroke volume	2.4	1.7	2.7	3.2	0	0
Cardiac output	-0.56*	-0.63*	-0.31	-0.08	0	2
Final						
Systolic pressure	-1.8	-2.6	-0.6	-3.0	0	0
Diastolic pressure	5.1*	3.6	6.0**	3.6	2	0
Pulse pressure	-6.9***	-6.3**	-6.7***	-6.7**	0	4
Heart rate	4.2	5.0	3.5	5.0	0	0
Stroke volume	2.7	0.8	1.4	-2.3	0	0
Cardiac output	0.56*	0.46	0.42	0.30	1	0

* P < 0.05.

** P < 0.01.

***P < 0.001.

These comparisons show that we have gained little by attempting to standardize the conditions of the test beyond those laid down for the original studies, of which Series O was a part.¹ The presence or absence of the fasting state appears to be the only factor of general importance.

Discussion: While many of the inferences to be drawn from the facts presented in the foregoing section have been mentioned, certain points deserve emphasis. In both Series F and Series E, we have found that the circulatory changes on smoking as measured in the first test are an unbiased estimate of the mean: although the mean of the results in repeated tests would give more information than a single test, nevertheless the first test is as accurate and informative as any other single test. This conclusion fulfills a most important requirement for a test which is to be useful as a screening procedure. Moreover, an analysis of variance showed that the intraindividual variations of systolic pressure, diastolic pressure, heart rate, and cardiac output are significantly smaller than the interindividual variations. This separation is an even more important requisite for a screening test, because it makes it possible with fair confidence to assign subjects to one of several broad groups according to the magnitude and direction of the response. These findings support the view previously formed that individual differences in the patterns of circulatory change on smoking have

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TABLE XVII. COMPARISON OF THE VARIANCE IN SERIES O AND E

MEASUREMENT	FIRST	SECOND	THIRD	FOURTH	FIFTH	SIXTH	SEVENTH	EIGHTH
Systolic pressure								
Control	0.284*	0.452	0.408	0.267**	0.321*	0.332*	0.241**	0.258*
Change	2.28	7.02*	1.13	0.500	0.417	0.385	0.649	4.89
Final	0.316	0.422	0.239**	0.194**	0.250**	0.192**	0.228**	0.267**
Diastolic pressure								
Control	0.629	1.22	0.826	0.962	1.16	0.917	0.336*	0.893
Change	0.625	0.730	1.04	0.543	0.870	0.893	0.763	2.38
Final	0.840	2.10	0.538	1.11	1.07	0.862	0.380	0.752
Pulse pressure								
Control	0.323*	0.448	0.719	0.373	0.690	0.625	0.906	0.571
Change	1.74	2.27	1.47	1.67	1.07	1.66	3.18	7.31*
Final	0.671	0.840	0.719	0.362	0.481	0.392	0.704	0.877
Heart rate								
Control	1.14	3.33	1.74	1.78	0.877	1.60	1.16	4.77
Change	0.667	0.617	0.334*	1.28	0.625	0.415	0.543	0.794
Final	0.633	1.44	0.971	1.45	1.42	2.03	1.11	2.19
Stroke volume								
Control	0.855	1.00	0.676	1.02	0.490	0.662	3.74	2.00
Change	0.283*	0.369	0.444	0.342*	0.578	0.549	0.383	1.77
Final	0.704	1.25	0.735	0.971	0.885	1.71	1.20	3.38
Cardiac output								
Control	0.943	1.04	0.909	1.14	1.20	0.971	1.00	2.10
Change	1.01	1.34	0.405	0.360*	1.52	1.82	1.72	1.06
Final	1.79	1.25	2.71	4.18	1.80	1.29	1.10	1.93

These figures were obtained by dividing the variance in Series O by those in Series E.

*P < 0.05.

**P < 0.01.

MEASUREMENT
Systolic pressure
Control
Rise
Final
Diastolic pressure
Control
Rise
Final
Pulse pressure
Control
Rise
Final
Heart rate
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Rise
Final
Stroke volume
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Cardiac output
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*P < 0.05.

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TABLE XVIII. COMPARISON OF THE MEANS IN SERIES O AND E

MEASUREMENT	FIRST	SECOND	THIRD	FOURTH	FIFTH	SIXTH	SEVENTH	EIGHTH
Systolic pressure								
Control	5.5	1.1	2.0	4.3	5.3	5.0	0.0	3.1
t	0.74	0.18	0.32	0.56	0.75	0.72	0.0	0.40
Rise	-1.6	3.4	0.6	0.4	-0.1	1.1	1.6	-0.9
t	0.89	2.79*	0.25	0.11	0.03	0.27	0.51	0.68
Final	3.9	4.5	2.5	4.7	5.2	6.0	1.5	2.2
t	0.53	0.70	0.30	0.50	0.63	0.64	0.17	0.27
Diastolic pressure								
Control	-1.4	-3.8	-4.4	-2.8	-2.4	-0.8	-2.8	-2.8
t	0.38	1.40	1.35	0.92	0.86	0.26	0.57	0.89
Rise	-0.6	1.4	2.6	0.3	-0.6	0.3	-2.9	-1.0
t	0.23	0.58	1.27	0.11	0.27	0.13	0.39	0.70
Final	-2.0	-2.3	-1.8	-2.5	-3.0	-0.5	-5.6	-3.8
t	0.56	0.97	0.41	0.80	0.94	0.14	1.08	1.01
Pulse pressure								
Control	6.9	4.9	6.4	7.1	7.7	5.7	2.7	5.9
t	1.01	0.84	1.38	1.12	1.62	1.15	0.61	1.14
Rise	-1.0	2.0	-2.0	0.1	0.4	0.8	4.5	0.1
t	0.48	1.01	0.83	0.04	0.14	0.35	2.60*	0.08
Final	5.9	6.9	4.4	7.2	8.2	6.6	7.2	6.0
t	1.13	1.47	0.87	1.03	1.35	0.98	1.42	1.31
Heart rate								
Control	-7.5	-3.9	-6.3	-6.2	-1.8	-7.4	-10.3	-4.7
t	1.69	1.38	1.71	1.70	0.36	1.94	2.34	1.89
Rise	1.2	1.7	0.1	3.6	0.5	2.1	2.9	1.7
t	0.34	0.46	0.02	1.36	0.14	0.47	0.74	0.52
Final	-6.3	-3.2	-6.2	-3.4	-1.3	-5.3	-7.4	-3.1
t	1.06	0.77	1.26	0.83	0.31	1.49	1.60	0.90
Stroke volume								
Control	7.0	6.5	4.4	3.1	5.7	6.4	4.7	2.7
t	0.97	0.97	0.52	0.47	0.61	0.79	1.22	0.55
Rise	3.3	1.2	1.3	1.5	-0.6	-4.2	-2.0	2.1
t	0.64	0.26	0.33	0.32	0.16	1.12	0.45	0.96
Final	10.3	7.7	5.7	4.7	5.1	2.2	2.5	4.8
t	1.19	1.16	0.67	0.63	0.66	0.38	0.37	1.10
Cardiac output								
Control	-0.35	-0.80	-0.37	-0.42	-0.09	-0.35	-0.79	-1.27
t	0.64	1.52	0.75	0.83	0.02	0.64	1.47	3.29*
Rise	0.36	0.36	0.10	0.52	0.17	0.07	0.26	0.43
t	1.14	1.29	0.20	1.01	0.65	0.29	1.04	1.39
Final	0.02	0.44	-0.26	0.10	0.28	-0.27	-0.53	0.16
t	0.04	0.79	0.65	0.30	0.59	0.49	0.90	0.35

In each instance the O value was subtracted from the E value.

*P < 0.05.

real meaning and are not artefacts due to variance. It is worth noting, however, that the scatter of the results within the individual varies appreciably from subject to subject, so that confidence limits may be safely applied to groups only.

On repeated tests, the changes after smoking which characterized the 6 subjects in Series E were, in descending order of effectiveness, heart rate, diastolic pressure, systolic pressure, and cardiac output. Changes in pulse pressure and stroke volume did not provide good separation for these 6, but might do so for

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larger and more representative groups. It was previously shown that there was quite good correlation between the pattern of response to smoking and the family history regarding hypertension or coronary disease.¹ As a group, subjects with parental hypertension showed a significantly greater rise in cardiac output than expected, while stroke volume and cardiac output were significantly smaller than expected among the offspring of parents with coronary disease. Changes in blood pressure and heart rate were not significantly different for these two small groups of subjects compared with those with normal parents, but levels of significance were approached in several instances. Accordingly, at present, cardiac output alone has been shown to have *both* important properties—that of giving satisfactory separation of subjects and of giving good correlation with family history. While the family history provides only indirect evidence of liability to future cardiovascular disease, it appears that, taken in conjunction with certain individual characteristics, it is helpful in pointing out susceptible persons.⁴⁻⁶ From the above facts it appears that change in cardiac output after smoking is likely to be the most valuable part of the ballistocardiographic smoking test in screening possible candidates for hypertension or coronary disease.

In this paper, we have attempted to discover whether there are any factors which influence the response to smoking, since elimination of such sources of variation might "sharpen" the results. The only factor which seems important is the presence or absence of the fasting state which appears to change the magnitude of the response. This raises the question as to whether the tests performed before breakfast are measuring the same tendencies as those performed before lunch, that is, will the subjects tested before breakfast show a proportionate change if tested before lunch? In order to answer this question, we have calculated correlation coefficients between the results of the four sets of tests. Table XIX indicates that heart rate and cardiac output show good agreement.

TABLE XIX. CORRELATION COEFFICIENTS BETWEEN THE RESPONSES TO SMOKING (SERIES F)

MEASUREMENT	IA AND IB	IIA AND IIB	IA AND IIA	IB AND IIB
Systolic pressure	+0.202	+0.669***	+0.267	+0.500**
Diastolic pressure	+0.144	+0.641***	+0.242	+0.443*
Pulse pressure	-0.211	+0.225	-0.174	+0.178
Heart rate	+0.670***	+0.726***	+0.776***	+0.497**
Stroke volume	+0.158	-0.005	+0.050	+0.296
Cardiac output	+0.596***	+0.660***	+0.582***	+0.525**

* P < 0.05.

** P < 0.01.

***P < 0.001.

This agreement is equally good whichever way the results are paired. Systolic and diastolic pressures show less satisfactory agreement. It seems legitimate then to conclude that the tests before breakfast and before lunch give similar information, but since the changes are on the average greater before breakfast, there is less danger that they will be obscured by experimental error. However, as shown in Table II, this increase in sensitivity has little practical effect on the

significance of the difference between those doing the tests, and accordingly, from the fasting tests, and

We have found no effect of smoking on the reproducibility of the test, that there is very little change in blood pressure in repeated smoking tests, which we have seen in increase in blood pressure to day.¹⁰ Unfortunately, in an earlier paper, we found that smoking are recorded with standard deviation and standard deviation

Fig. 4.—The increase in blood pressure in 10 healthy subjects in mm. Hg. The solid line shows the mean (at rest) and the three vertical lines show the standard deviation (at rest) and finishing was lower when the subjects were fasting figures (at rest) (Ruosteenoja, R. J. Med. Exper. Fenn)

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	+0.443*	
	+0.178	
**	+0.497**	
	+0.296	
*	+0.525**	

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significance of the change after smoking, and there is little further difference between those done before lunch and those done at random (Table XVI). Accordingly, from our studies we conclude that there is little to be gained from fasting tests, and that tests performed at random times of day are satisfactory.

We have found few papers that deal with the quantitative aspects of the effect of smoking on the normal ballistocardiogram, and none which reported on the reproducibility of results.⁷⁻⁹ Indeed, a search of the literature has shown that there is very little information on the reproducibility of the results obtained in repeated smoking tests of any type on the individual. The only reference which we have encountered is in a paper by Roth, in 1956, who writes, "... the increase in blood pressure and pulse rate during smoking varied little from day to day."¹⁰ Unfortunately, she provides no figures or analysis of her results. In an earlier paper, however, the changes in blood pressure and heart rate after smoking are recorded for nine tests on one normal subject.¹¹ Calculation of standard deviations in this one case gave excellent agreement with the mean standard deviations in our Table X.

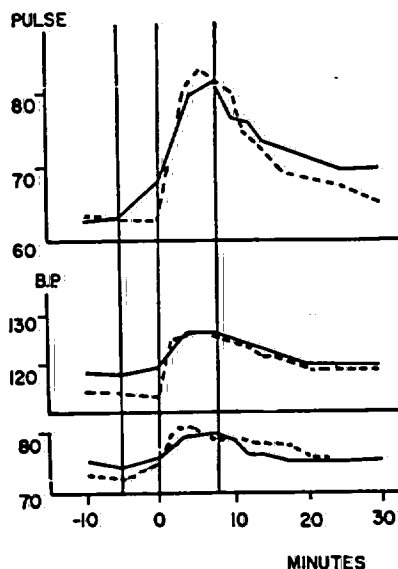


Fig. 4.—The influence of the fasting state on the circulatory response to smoking: average changes in 10 healthy subjects. From above down: pulse rate in beats per minute, systolic and diastolic pressure in mm. Hg. The broken line indicates the average of smoking tests performed in the fasting state, the solid line shows the average of tests in which a meal of pea soup immediately preceded smoking. The three vertical lines denote the times of beginning the meal (—5 minutes) starting to smoke (0 minutes) and finishing smoking (7½ minutes). It will be seen that in all measurements the value at 0 minutes was lower when the subjects were fasting, but they showed a bigger rise on smoking and the "final" fasting figures (at 7½ minutes) were almost identical with the nonfasting values. (Modified from Ruosteenoja, R.: Effect of Food Intake on the Cardiovascular Response to Cigarette Smoking, *Ann. Med. Exper. Fenn.* 33:320, 1955.)

Ruosteenoja compared the circulatory changes in 10 students aged 20 to 25 years (5 regular and 5 occasional smokers) under three sets of circumstances: immediately after a meal, on smoking after a meal, and on smoking after 15

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hours' fasting.¹² In all other respects the test conditions were kept constant. These experiments show that there was a greater rise in skin temperature, systolic and diastolic pressure, and in pulse rate if the subject was fasting than there was after a meal (Fig. 4). In contrast to changes in skin temperature, which were highly significant, the differences in blood pressure and pulse rate were just significant at the 5 per cent level of probability. Although he makes no comment on the point, it appears from his diagrams, that the control values immediately before smoking were lower in the fasting state, and the highest figures after smoking were about the same whether or not the subjects were fasting. These data, therefore, agree nicely with our concept of the ceiling phenomenon which we have postulated as a result of the findings in Series F.

In a somewhat older group, Roth and Sheard found smaller differences in blood pressure and heart rate than we did when the changes on smoking in the fasting and nonfasting states were compared.¹³ The discrepancies between their results and ours, however, do not seem great and may well result from differences in age and other characteristics of the subjects studied, as well as in the timing of the observations in relation to the last meal.

SUMMARY

1. A total of 245 ballistocardiographic smoking tests were done on healthy male medical students, all of them smokers. Sixty-nine subjects (Series O) had one test each at a random time in the day. To test the reproducibility of results 32 men had four tests each, two before breakfast and two before lunch (Series F); and 6 subjects had eight tests each under circumstances kept as standard as possible in every way (Series E).

2. The findings on a single smoking test in 113 students previously described, were essentially unchanged when the nonsmokers and female students were excluded, leaving the 69 subjects of Series O.

3. In 32 male smokers (Series F), smoking a cigarette before lunch produced results similar to those in Series O: a highly significant mean rise in systolic and diastolic pressure, heart rate, and cardiac output, and a fall in stroke volume. Pulse pressure was little affected. Compared with these tests, those performed before breakfast showed lower control readings, with a complementary increase in the response to smoking, so that the final figures after smoking were remarkably constant. Results were essentially the same on a second day. Heavier smokers tended to have lower control values and a bigger response to smoking. Exercise habits influenced the results little.

4. In the eight tests repeated on each of 6 subjects, the first test appeared to be an unbiased estimate of the mean. The reproducibility of the results was calculated, and it proved to be an individual characteristic, that is, mean confidence limits are of value in groups of subjects only. Analysis of variance shows that the change in systolic and diastolic pressures, heart rate, and cardiac output after smoking are likely to prove of value in classifying the subjects, but that for the other two measurements, the "overlap" of individual variation appears to be too great.

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Heart
9. Simon, D. L.
Circula
raphy,
10. Roth, G. M.
Med. 3
11. Roth, G. M.
Intrave
Rate, 6
J.A.M.
12. Ruosteenoja,
Smoki
13. Roth, G. M.
Oral A
J. 33:6

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5. Comparison of the three series of tests revealed no great differences. The results in Series O, Series E, and the tests before lunch in Series F were substantially the same. The findings in Series F before breakfast were the only exceptions: in the main, control readings were lower and the response to smoking proportionately greater at that time than in any other series of tests. However, this enhancement of response seems of little practical value.

6. From these findings it appears that the ballistocardiographic smoking test, in which blood pressure, heart rate, stroke volume, and cardiac output are measured, gives results which are sufficiently reproducible for use as a screening test to classify young adults according to their patterns of circulatory reactivity. The results were materially unchanged by attempts to standardize further the test conditions, except that on the average, the response was more marked in the fasting state.

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1. Thomas, C. B., Bateman, J. L., Lindberg, E. F., and Bornhold, H. J.: Observations on the Individual Effects of Smoking on the Blood Pressure, Heart Rate, Stroke Volume and Cardiac Output of Healthy Young Adults, *Ann. Int. Med.* 44:874, 1956.
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4. Bolt, W., and Lew, E. A.: Prognostic Value of Life Insurance Mortality Investigations, *J.A.M.A.* 160:736, 1956.
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#203

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N.Y.

(Cf. #89R1
Activated 10/1/55
Renewed 9/1/56)

Application For Research Grant

Date: April 15, 1958

1. Name of Investigator: Dr. Caroline Bedell Thomas
2. Title: Associate Professor of Medicine
3. Institution & Address: The Johns Hopkins School of Medicine
710 North Washington Street
Baltimore 5, Maryland
4. Project or Subject:
 - a. Studies of Genetic Differences between Smokers and Nonsmokers
 - b. Studies of Psychological Differences between Smokers and Nonsmokers as Shown by Comparison of Figure Drawings.
5. Detailed Plan of Procedure (Use reverse side if additional space is needed): See Part C attached;
Plans for the future. (Part A: Progress in special smoking studies.
Part B: Progress report for National Heart Institute Grant
Appendix I: new paper on familial and epidemiologic aspects of coronary disease and hypertension.
Appendix II: new paper on the ballistocardiographic smoking test.)

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6. Budget Plan:

For detailed explanation
see Part C p. 4

Salaries

\$ 5,500.00

Expendable Supplies

222.25

Permanent Equipment

1,500.00

Overhead

4,278.75

Other

11,500.00

Total

7. Anticipated Duration of Work:

Two years

8. Facilities and Staff Available:

Dr. C. Lockard Conley and Dr. Julius R. Krevans, who are in charge of the blood bank, have given us permission to make use of blood bank donors and blood group data in the manner indicated. There is ample work space available in conjunction with our major project. The staff collaborators have been indicated above. A part-time statistician will be available.

9. Additional Requirements: None

10. Additional Information (Including relation of work to other projects and other sources of supply):

The aims of the project outlined above are in harmony with those of Grant H-1891 (C4) entitled "Presursors of Hypertension and Coronary Artery Disease" awarded by the National Heart Institute. The funds from that source do not include most of the items covered by the budget given above. Where similar items exist in each of the two budgets, it is because the budget from Grant H-1891 (C4) is insufficient to meet the total expense of a given item, and the two budgets will be used in such a way that they supplement each other.

Signature /s/ Caroline Dedell Thomas
Director of Project

/s/ Samuel B. Asper, Jr. Associate
Business Officer of the Institution Dean

1003537140

Application For Research Grant

Date: May 9, 1955

1. Name of Investigator: **Dr. Caroline Bedell Thomas**
2. Title: **Associate Professor of Medicine**
3. Institution & Address: **The Johns Hopkins School of Medicine
710 North Washington Street
Baltimore 5, Maryland**
4. Project or Subject: **The significance of different individual patterns of circulatory response to cigarette smoking.**

5. Detailed Plan of Procedure (Use reverse side if additional space is needed): **As part of a long term study of the precursors of hypertension and coronary artery disease, using the Johns Hopkins medical students as subjects, we have made preliminary studies of the circulatory patterns produced by cigarette smoking. With the subject lying on a Starr-type ballistocardiograph, the systolic pressure, diastolic pressure, pulse pressure, heart rate, stroke volume, cardiac output and cardiac index were measured before and after smoking. Analysis of tests on the first 113 subjects showed:**

1. Whereas the overall mean change after smoking a single cigarette was not great, striking variations were found in individual patterns not only in the degree but in the direction of change (Figures 1 and 2).
2. Where one variable showed a marked deviation in a given direction, the other variables tended to fall into a characteristic pattern (figure 3).
3. When subjects were classified according to age, sex, body weight, blood cholesterol levels, smoking habits or as to whether or not parental history of hypertension and/or coronary artery disease is present, our results suggest that the pattern of response to smoking may be more closely linked to parental history than to the other factors mentioned (Figures 4 and 5.)

These suggestive preliminary findings are now under detailed statistical analysis. Before their meaning is clear, much more work needs to be done both by studying larger groups of subjects with different types of parental history and by certain other intensive studies. The assistance of a part-time Physiological Fellow is necessary to accomplish these aims.

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6. Budget Plan:

Salaries	\$ 1,000.00
Expendable Supplies	100.00
Permanent Equipment	0.00
Overhead	280.00
Other	300.00
Total	\$ 1,680.00

7. Anticipated Duration of Work:

One year, beginning in the fall of 1955

8. Facilities and Staff Available:

There is a Starr-type ballistocardiograph and a double-channel Sanborn Viso-cardiette available as well as ample work space in conjunction with our major project, access to our records, and secretarial assistance. The work will be under my immediate direction.

9. Additional Requirements:

In addition to the salary item for the Fellow and \$100 to cover the cost of certain pharmacological products to be used in connection with his work, the \$300 item under "other" will provide a small weekly sum for ballistocardiographic measurements and calculations to be carried out by a trained medical student paid at an hourly rate. (See Appendix A and B)

10. Additional Information (Including relation of work to other projects and other sources of supply):

The smoking test studies are to be carried out in conjunction with Grant H-1891 (c) under the Public Health Service, National Institutes of Health, entitled "A Study of The Precursors of Hypertension and Coronary Artery Disease." That budget does not provide for the items here requested.

Signature /s/ Caroline Bedell Thomas
Director of Project

/s/ Philip Bard
Business Officer of the Institution
Dean & Budget Officer

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Figure 1

The pattern of the mean circulatory changes of
113 healthy young adults in response to smoking one cigarette.

SP = systolic pressure

DP = diastolic pressure

PP = pulse pressure

HR = heart rate

SV = stroke volume

CO = cardiac output

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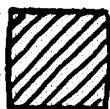
Figure 2

Six different individual patterns of circulatory response to smoking. Each pattern represents the percentage changes in six variables shown by a single subject immediately after smoking one cigarette compared with his own control values. (Legend as in Figure 1).

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Figure 3

Patterns of circulatory response to smoking among twelve selected groups. Each pattern represents the mean changes for the ten subjects having the greatest unidirectional change of the index determinant as indicated in the subtitle.



■ index determinant



■ associated determinants

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Figure 4

The patterns of circulatory response to smoking
among smokers versus non-smokers.

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Figure 3

The patterns of circulatory response to smoking among subjects with parental history of hypertension versus subjects with normal parents and subjects with parental history of coronary artery disease.

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TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

Application For Research Grant

/RENEWAL

Date:

April 12, 1956

1. Name of Investigator:

Dr. Caroline Bedell Thomas

2. Title:

Associate Professor of Medicine

3. Institution

& Address:

The Johns Hopkins School of Medicine
710 North Washington Street
Baltimore 5, Maryland

4. Project or Subject:

The Significance of Individual Smoking Patterns in Healthy
Young Adults

Part a. Comparison of smokers and nonsmokers in regard to 1) family history of hypertension and/or coronary artery disease; 2) physiologic characteristics; and 3) psychologic traits.

Part b. Ballistocardiographic studies of the circulatory response to smoking, with
5. Detailed analysis of differences associated with family history, physiologic and
psychologic traits.

Analysis of differences

Part a) Since the initiation of a long-term study of Possible Precursors of Hypertension and Coronary Artery Disease ten years ago, the smoking habits of over 700 Johns Hopkins medical students have been determined. Detailed family histories, measurements of blood pressure and heart rate at rest and under standardized forms of stress, eosinophil counts, blood cholesterol levels, individual Rorschach tests, and surveys of the personal background and habits have also been obtained on the same group of subjects. Follow-up studies are in progress. It is our plan to subdivide the population of subjects into groups according to amount, type and duration of smoking and to carry out a statistical analysis of whatever differences are to be found between these groups in regard to each type of information obtained in the long-term study.

Coding and tabulating of the fix pertinent data for transferral to IBM punch cards is under way. The first set of cards has just been completed and tabulation of the second set of cards is nearly ready for punching. It is estimated that the tabulation and punching of the basic data outlined in the semi-annual progress report, Part II, pages 5 and 6, will be completed by September 30, 1957, including all information obtained on the classes of 1948 through 1957, and partial information on the classes of 1958 and 1959 which will still be in medical school at that time. (Data is collected throughout the four academic years.)

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Detailed Plan of Procedure - Cont'd.

The statistical analysis of this large volume of data and writing it up for publication will take a minimum of three years after the basic tables are on hand. The first paper, which it is hoped can be completed in 1956-1957, is to be an inquiry into possible associations between family history of hypertension and/or coronary artery disease and the smoking habits of the subjects themselves, in which Mrs. Bernice Cohen and Miss Mary Burke will collaborate with the director.

Part b) Ballistocardiographic studies of individual patterns of circulatory response to cigarette smoking are to be continued along the lines described in the semiannual progress report, Part 1, pages 1-5. A part-time Fellow well-trained in physiologic experimental procedure is available for the year beginning on July 1, 1956, who can implement this work in a way which has not been possible this year with a Fellow without such research training. By adding to the numbers of subjects during the coming year and by making additional observations designed to cross-check and further illuminate our initial findings, it is thought that we shall have a much better comprehension of the meaning of individual variations in the circulatory effects of smoking.

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6. Budget Plan: October 1, 1956 - September 30, 1957

Fellow; two statistical clerks }
student assistants; director. }

Salaries
Expendable Supplies
Permanent Equipment
Overhead
Other

\$8,400
300.
000
1,924
720
\$ 11,544.

Services and rental, IBM equip- }
ment. }

Total

7. Anticipated Duration of Work: Three years.

8. Facilities and Staff Available: There is ample work space in conjunction with our major project including a separate laboratory equipped with a Starr-type ballistogardiograph and a double-channel Sanborn Viso-Cardiette.

The entire project will be under my direct supervision, and will be assisted by the secretary and the physiological technician of the long-term project where required.

9. Additional Requirements: Continuation of the advice and counsel of Miss Mary Burke, Statistician to the Tobacco Industry Research Committee.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The aims of the project outlined above are in harmony with those of Grant H-1891 (C2) entitled "Precursors of Hypertension and Coronary Artery Disease" awarded by the National Heart Institute. The funds from that source do not include many of the items covered by the budget given above. Where similar items exist in each of the two budgets, it is because the budget from Grant H-1891 (C) is insufficient to meet the total expenses of a given item, and the two budgets will be used in such a way that they supplement each other.

s/s/
Signature Caroline Bedell Thomas
Director of Project

s/s/ (Associate Dean) Institution
Business Office of the

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CONFIDENTIAL

TIRC Grant #89

Progress Report #1

Dr. Caroline Bedell Thomas
The John Hopkins School of Medicine

April 11, 1956

Individual Patterns of Circulatory
Response to Cigarette Smoking:
Ballistocardiographic Studies

Since the original application to the Tobacco Industry Research Committee a year ago for assistance in a project concerning individual patterns of circulatory response to cigarette smoking, the basis of our collaboration has been broadened to include other objectives and the support we have received has been of two kinds. First, a research grant of \$4,000 has supplemented funds received from the National Institutes of Health for grant H-1891(c) entitled "A Study of the Precursors of Hypertension and Coronary Artery Disease". The grant from the Tobacco Industry Research Committee has provided the salaries of a part-time Fellow, Dr. Amirali Kassam, and of two clerical assistants. It has also covered most of the running expenses incurred while carrying out ballistocardiographic smoking tests on the Johns Hopkins medical students during the current academic year. Secondly, our need for continuing counsel and advice concerning the statistical aspects of the combined study has been met by the assignment of Miss Mary Burke, the Statistician of the Tobacco Industry Research Committee, to our project three days a week.

The work resulting from this support by the Committee is as follows:

1. Physiologic studies on the effects of smoking.

A. The first paper dealing with the ballistocardiographic smoking test, entitled "Observations on the Individual Effects of Smoking on the Blood Pressure, Heart Rate, Stroke Volume and Cardiac Output of Healthy Young Adults" was submitted for publication in December and is to appear in the May issue of the Annals of Internal Medicine. Although work on this paper was essentially complete in October, several conferences with Miss Burke were most helpful in the final orientation of the statistical findings to the paper as a whole. A brief summary of the results to be published in this paper is given in the Progress Report for 1955-1956 to the National Institutes of Health. (See Appendix A)

B. The effect of smoking upon certain other ballistocardiographic measurements has been studied with the assistance of Dr. Kassam. Using the same 113 smoking tests, the JK/IJ ratio and the ratio between the small (S) and large (L) IJ waves, which occur in expiration and inspiration respectively, have been analyzed. The findings are given in Appendix B, Tables I and II.

1. It will be seen that smoking produced no substantial change in most of these ratios, either when the group as a whole is considered or when the group is subdivided according to a) sex, b) smoking habits or c) parental history of hypertension or coronary artery disease. The only statistically significant changes are indicated in Tables I and II, Appendix B.
2. In particular, there was no significant difference between smokers and nonsmokers in the effect of smoking on the JK/IJ ratio.
3. The smokers, who had the higher $IJ_S / IJ_L \times 100$ ratio, showed a significant decrease after smoking, while the nonsmokers did not. It is possible

that this finding may reflect an alteration in the respiratory pattern associated with the inhalation of tobacco smoke.

4. The women showed the most pronounced deepening of the K wave after smoking of any group, while the subjects with hypertensive parental history showed a decrease in the size of the K wave after smoking.

5. The offspring of hypertensive parents had the largest mean value (1.15) for the JK/IJ ratio during the control period, indicating that the K waves were most prominent in that group. It is known that deep K waves are characteristic of the hypertensive patient. Our finding is of interest, therefore, because it suggests that the ballistic pattern characterized by deep K waves may precede the onset of elevated blood pressure.

C. Studies concerning the effect of smoking on the ballistocardiographic form of healthy young adults.

1. The first 225 smoking tests carried out on medical students 21-35 years of age have been reviewed in regard to the normality of ballistocardiographic form. Of this number, 18 subjects, or 8 percent showed borderline or early abnormal changes. (Table I, Appendix C). In three individuals such changes were present before smoking; one of these deteriorated further after the first cigarette. In thirteen subjects normal form in the control tracing became borderline or abnormal after the first cigarette. In two, these changes followed the second cigarette.

2. The borderline changes in ballistocardiographic form consisted chiefly of rounding, blunting or notching of the J and K waves and the occasional presence of early M waves in successive cycles. In the records interpreted as "abnormal", these changes were somewhat more pronounced, the small complexes had decreased in size and become somewhat irregular. In two individuals the record was distorted by tumultuous action with exaggerated waves during tachycardia after smoking. In all but two records (indicated by asterisks in Table I, Appendix C) the abnormal form was slight enough to permit measurement of the complexes.

3. Certain characteristics of the 18 subjects with alterations in ballistocardiographic form have been summarized in Tables I and II, Appendix C. In comparison with an unselected total population of subjects, these 18 subjects show (Table III, Appendix C):

- a. A higher proportion of smokers and former smokers.
- b. A smaller proportion of nonsmokers.
- c. A higher proportion of parental hypertension and/or coronary artery disease.
- d. A higher mean blood cholesterol level.
- e. A higher proportion of individuals who are more than 20 pounds overweight.

In addition to these differences, the mesomorphic build was present in most of the 18 subjects under consideration. The incidence of mesomorphy is undoubtedly much higher than that found among the medical students as a whole, but we have not yet analyzed our general data in this regard.

In summary, the 18 subjects with ballistocardiographic changes are somewhat different from their classmates in a number of ways. The traits which characterize them are those which are thought to be commonly associated with coronary artery disease,

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namely, family history of cardiovascular disease, elevated blood cholesterol levels, mesomorphy and obesity. The majority of the 18 have been smokers for a period of years. Our present data does not allow us to do more than point out the associations. We do not yet know the cause of the deterioration of the ballistocardiographic form in these individuals.

D. Studies on the effect of smoking on the same individual at different times of day and on different days are in progress. The design of the experiment is as follows: the participants are all smokers in the class of 1958. The subject comes to the laboratory at eight in the morning, before breakfast and before smoking, and a one-cigarette smoking test is performed. On the same day the subject has another such test at one o'clock, after morning classes and before lunch. The subjects are allowed to smoke if they please up to noon; if they have smoked, the number of cigarettes is recorded. The two tests are performed by the same examiner. Later in the year the student returns for two more smoking tests on the same day, one before breakfast and one before lunch, as before. In this way each subject has four smoking tests on two days at two different times of day. Twenty-six subjects have completed the first two tests, and we are in process of repeating the tests for the second half of the study.

II Compilation of Data reviewed in regard to the correlation of ballistocardiographic

A. The following portions of the combined study are in the process of being coded for IBM punch cards:

1. Card 1. General information

- a. Study identification number; other identifying material.
- b. Age; sex; birthplace; ancestry
- c. Parental history of hypertension, coronary artery disease, obesity and diabetes.
- d. Summary of smoking habits.
- e. Percentage of over-or underweight.
- f. Status of subject at time of Rorschach test
- g. Total number of responses to Rorschach test
- h. Summary of academic standing.

2. Card 2. Studies of blood pressure and heart rate.

- a. Under resting conditions.
- b. As affected by the cold pressor test.
- c. As affected by the double Master exercise test.
- d. As affected by the anoxemia test.
- e. As affected by the smoking test.

3. Card 3. Analysis of smoking habits.

4. Card 4. Analysis of cholesterol data

5. Card 5. Analysis of the Rorschach data

6. Card 6. Analysis of other habit survey data

7. Card 7. Analysis of other physiologic data

on the 18 subjects under consideration. The incidence of mesomorphy is un-

The first set of cards has been completed and frequency distribution tables and crosscorrelations between various items are now being analyzed.

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Appendix A

A. During the current year we have:

1. Continued to collect genetic, physiologic, psychologic and metabolic data on the Johns Hopkins medical students. Microfiled data from 1954-1955.
2. Sent out five-year follow-up questionnaires to the class of 1951, the fourth class thus studied.
3. Completed a paper concerned with smoking tests given on the ballistocardiograph. (See publications sheet) Statistically significant changes occurred in systolic pressure, diastolic pressure, pulse pressure, heart rate, stroke volume, cardiac output and cardiac index after smoking once cigarette. The direction and degree of change after smoking varied greatly from subject to subject, so that striking individual differences in circulatory patterns were found. On the average, students with parental hypertension, whose control cardiac output and cardiac index were large to begin with, showed more than twice as great an increase in cardiac output and cardiac index as did subjects with negative parents. Likewise, subjects with a parental history of coronary artery disease showed very little increase in cardiac output and cardiac index in contrast to subjects with negative parents.
4. Completed the statistical analysis of cholesterol data on 612 subjects, with particular regard to sources of variation such as age and sex of subject, and the reliability of laboratory method. This basic paper is now being written.
5. Analyzed the relationship of parental history to the total blood cholesterol of the subject. This paper is to appear as a companion to, or immediately after, the previous one. (See 4)
6. Completed plans for tabulation of the study as a whole on IBM cards and taken first steps to set this process in motion.
7. Received a supplementary grant of \$4,000 from the American Tobacco Industry Research Committee on October 1, 1955, which has enabled us to carry out duplicate smoking tests on the class of 1958 with the assistance of a part-time Fellow, and to obtain additional clerical assistance for the tabulation of the data on smoking habits of more than 800 subjects for analysis and cross-correlation with other aspects of the study.

B. The Tobacco Industry Research Committee has loaned us their statistician, Miss Mary Burke, three days a week since the beginning of October, as a consultant. This temporary addition to our staff has been most valuable.

C. Mrs. Bernice H. Cohen is associated with the study part-time this year while continuing her studies for a Ph.D. degree in Human Genetics, under a Predoctoral Fellowship from the National Institutes of Health.

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Number of subjects		JK/IJ Ratio					IJ _s /IJ _L X 100 Ratio				
		Total	Men	Women	Smokers	Nonsmokers	Total	Men	Women	Smokers	Nonsmokers
		113	103	10	81	32	113	103	10	81	32
Control Values	Mean	1.11	1.11	1.09 ^A	1.11	1.12	72	72	74	74 ^{B,C}	68 ^C
	Lowest	0.88	0.88	0.91	0.91	0.88	45	45	50	52	45
	Highest	1.40	1.40	1.24	1.40	1.36	96	89	96	96	87
After First Cigarette	Mean	1.11	1.11	1.17 ^A	1.11	1.13	70	70	69	70 ^B	69
	Lowest	0.92	0.92	1.06	0.92	0.93	47	47	47	52	47
	Highest	1.52	1.52	1.27	1.50	1.52	84	84	81	84	83
After Second Cigarette	Mean	1.12	1.11 ²	1.17	1.12 ³	1.12	70	70 ²	72	70 ³	70
	Lowest	0.77 ¹	0.77 ²	1.03	0.77 ³	1.02	43 ¹	43 ²	43	49 ³	43
	Highest	1.54 ¹	1.54 ²	1.30	1.54 ³	1.50	97 ¹	91 ²	97	97 ³	83

- 1 Out of 113, 110 had second cigarette test.
- 2 Out of 103, 100 men had second cigarette test.
- 3 Out of 81, 78 had second cigarette test.

Statistically significant comparisons:

- a. Change with smoking $p < .01$
- b. Change with smoking $p < .01$
- c. Difference between smokers and nonsmokers $p < .01$

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		JK/IJ Ratio				IJ _s /IJ _L X 100 Ratio			
Number of Subjects		H-C-	H+C-	H-C+	H+C+	H-C-	H+C-	H-C+	H+C+
		49	12	11	9	49	12	11	9
Control Values	Mean	1.11	1.15 ^A	1.12	1.09	73	72	74	70
	Lowest	0.88	1.07	0.98	1.03	53	50	58	52
	Highest	1.36	1.21	1.25	1.18	96	87	86	83
After First Cigarette	Mean	1.13	1.08 ^A	1.14	1.10	71	70	71	68
	Lowest	0.93	0.95	1.00	0.97	52	47	53	56
	Highest	1.52	1.19	1.36	1.24	83	82	87	75
After Second Cigarette	Mean	1.12	1.12 [✓]	1.14	1.12	72 [✓]	68 [✓]	68	69
	Lowest	0.77	0.95 [✓]	0.93	1.00	49 [✓]	43 [✓]	58	52
	Highest	1.50	1.21 [✓]	1.40	1.23	97 [✓]	81 [✓]	81	79

1 Out of 49, 47 had second cigarette test.

12 Out of 12, 11 had second cigarette test.

Statistically significant comparisons:

a. Change with smoking p < .01

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Age, Sex, and Smoking Habits of Eighteen Subjects showing Borderline or Abnormal Ballistocardiographic Form												
Subject No.	Sex	Age	Cigarette			Smoking Habits	Years of Smoking	Amount Smoked Daily			Remarks	
			Control	After 1st Cigarette				Cigarettes	Pipes	Cigars		
				A	B							
55204	F	28	A	A	A	S	8	10	0	0	Vomited after 2nd cigarette	
53137	M	28	B	A	A	S	10	15	0	0		
53162	M	28	B	B	B	S	12	30	0	0		
58119	M	32	N	A*	A*	S/N	8	20/0	0	0	Nausea, pallor, sweating and weakness after 1st cigarette	
58174	M	22	N	A	A	S	4	20-40	+	+	Smokes chiefly on weekends	
57134	M	23	N	A*	A*	S	4	12-15	0	0	Marked tachycardia	
53128	M	27	N	A	B	S	8	15	0	0		
58148	M	22	N	B	B	S	0	0	0	0		
58114	M	22	N	B	B	S	0	0	0	0		
56151	M	23	N	B	B	S	0	0	0	0		
53176	M	26	N	B	B	S	7	20	0	0		
56160	M	23	N	B	B	S	8	30	0	0		
58169	M	23	N	B	B	S	7	0	12	0		
56211	F	27	N	B	B	S/Occ.	6	+/Occ.	+/0	0		
58238	F	23	N	B	B	S	5	10	0	0		
53123	M	29	N	B	B	S/Occ.	10	20/0	0	0		
53108	M	25	N	N	N	S	5	0	4-5	1		
53142	M	26	N	N	N	S	5	0	+	0		

N equals normal form
 B equals borderline, abnormal form
 A equals abnormal form
 * equals could not be measured
 ** equals test discontinued

S equals Smoker
 N equals Nonsmoker
 Occ. equals occasional smoker
 S/N equals now nonsmoker, previously smoker

+ equals smokes, amount unspecified

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TABLE II

Other Characteristics of Eighteen Subjects showing Borderline or Abnormal Form

Subject No.	Sex	Age	First Cholesterol	Parental H/C	Height	Weight	Pounds Overweight
55204	F	28	160	+	69	173	+ 24
53137	M	28	330	+	75	20	+ 13
53162	M	28	295	-	74	193	+ 11
58119	M	32	244	-	67	136	-13
58174	M	22	265	*	71	227	+69
57134	M	23	250	*	73	174	+ 5
53128	M	27	360	+	72	181	+ 12
58148	M	22	310	*	67	152	+ 10
58114	M	22	266	+	69	183	+ 33
56151	M	23	298	+	69	174	+ 23
53176	M	26	288	+	74	162	-18
56160	M	23	207	?	72	208	+ 44
58169	M	23	203	*	74	162	-13
56211	F	27	222	-	68	117	-28
58238	F	23	222	+	65	130	0
53123	M	29	185	+	73	230	+ 53
53108	M	25	243	-	73	291	+118
53142	M	26	258	+	71	173	+ 10

* equals neither parent over 50 years old
H/C equals No hypertension and/or coronary artery disease

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TIRC Grant #89

Appendix C

TABLE III

Characteristic	18 subjects with changes in BCG form	Unselected Subjects	
			N
Regular smoker	67%	50%	260
Nonsmoker	11%	32%	260
Mean cholesterol level (in mgm s. percent)	256	229	612
Parental history of hypertension and/or coronary artery disease	50%	28%	260
Overweight by 20 lbs. or more	39%	12%	257

1003537159

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

Application For Research Grant

#163

(Compare #89
Activated Oct. 1, 1955
Renewed Aug. 1, 1956
Supplemented Oct. 1, '56
Present anniversary date
Aug. 31, 1957

Date:

July 22, 1957

1. Name of Investigator:

Dr. Caroline Badell Thomas, M.D.

2. Title:

Associate Professor of Medicine

3. Institution

& Address:

The Johns Hopkins School of Medicine
710 North Washington Street
Baltimore 5, Maryland

4. Project or Subject:

Studies on Smoking in Healthy Young Adults.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

It is proposed to study the effects of smoking, both acute and chronic, upon healthy young adults, to ascertain in what ways smokers as a group differ from nonsmokers, to observe the different individual circulatory reaction patterns appearing in a ballistocardiographic smoking test, and to attempt to modify those reaction patterns through the use of pharmacological substances in order to understand better the physiological mechanisms involved. The Johns Hopkins medical students are to be used as subjects for the smoking studies. The facilities and extensive data of the long-term study, in progress over ten years, of the precursors of hypertension and/or coronary heart disease supported by the National Heart Institute provide the basic materials needed. The large amount of work and money already invested in that study can be utilized effectively in obtaining answers to the question: Who is a smoker? Likewise, the follow-up program of the cardiovascular study, designed to determine just which subjects develop hypertension or coronary heart disease at an early age, may ultimately can make a valuable contribution to the smoking studies.

- a. Comparison of smokers and nonsmokers among the Johns Hopkins medical students in regard to 1) family history of hypertension and/or coronary artery disease; 2) physiological characteristics; 3) psychological traits; and 4) educational, religious and family background.

During the past year many of these comparisons have been undertaken, and some of the statistical analyses are nearly complete (see Progress Note, Section I for Grant #89). However, much more remains to be done before these preliminary results are ready for publication. In some instances, it may prove desirable to add data now on hand from the classes of 1958, 1959 and 1960. Tabulations presented in the Progress Report are based on information from the classes of 1948 through 1957. More statistical computations need to be done on many aspects of the physiological and psychological

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studies, so far only touched upon. Finally, many portions of the data are awaiting analysis. These include:

- (1) Relationship of family history of obesity and diabetes to smoking habits of students.
- (2) Correlation of smoking habits with:
 - (a) health history of the students themselves.
 - (b) the effect of oximeter-controlled anoxemia.
 - (c) the circulating eosinophil count as a measure of adrenal cortical activity.
 - (d) body build, using the ponderal index, height / $\sqrt[3]{\text{weight}}$ as well as clinical observations.
 - (e) habits of sleeping, eating, drinking, self-medication, work, exercise and recreation.
 - (f) birthplace and ancestral background.
 - (g) educational background, including an analysis of whether the subjects attended private schools, boarding or day schools.
 - (h) religious affiliation and training.
 - (i) occupation of parents.
 - (j) relationship of subject with father and mother and of parents with each other.
 - (k) factors leading to insecurity in childhood, including divorce of parents and death or chronic illness of a parent.

A good deal of mature consideration will be needed as to the best way to write up and publish this material in view of the current prominence given to research in regard to smoking by the popular press. Even though many of the preliminary findings described in the Progress Note are statistically significant, the numbers of subjects involved are not large, and we certainly want to avoid premature publicity.

- (b) Ballistocardiographic studies of individual circulatory patterns of response to smoking.

During the past two years we have demonstrated the satisfactory reproducibility of the ballistocardiographic smoking test in a given individual. These studies have laid the ground work for those we are now undertaking: within the past few weeks, we have started on a new series of ballistocardiographic smoking studies designed to elucidate the differences in individual patterns of response to smoking. Through the use of various pharmacologic substances it is hoped that the roles played, in human beings, by the sympathetic nervous system, the adrenal gland and other physiologic mechanisms may be better understood. At present the effects of intravenous injections of small doses of hexamethonium are being compared with similar injections of ~~hexamethonium~~ normal salt solution. The same subjects are to have repeated tests, and the dosage is to be gradually increased with each test until a clear cut end point is obtained.

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Other substances under consideration for study include adrenalin, nor-adrenalin, Wyamin, atropine, cortisone, ACTH and alcohol. This approach is a difficult one, particularly when it is remembered that elevation of heart rate beyond a certain point makes it difficult or impossible to make accurate ballistocardiographic measurements of stroke volume. It is our intention to keep the dosages small and to search for leads which may open up new areas of study, at least in regard to some of the substances employed.

c. Studies using circulatory ballistocardiographic patterns as the basis for classification and comparison.

Now that over 300 subjects have had ballistocardiographic smoking tests, students can be classified in new ways according to their physiologic responses to smoking. Our first paper on the smoking test indicated that different degrees of increase or decrease in cardiac output after smoking a single cigarette were significantly linked with differences in heritage in regard to hypertension or coronary heart disease (1). Comparisons of the physiologic, metabolic and psychologic attributes of those who are hyperreactive or nonreactive to smoking may prove to be much more revealing than the comparisons of smokers with nonsmokers, particularly in regard to psychological differences.

d. Further studies of the relationship of smoking to cholesterol levels.

In view of the finding that smokers have higher average cholesterol levels than nonsmokers (see Progress Note Section I D), it may be possible during the coming year to throw more light on this problem by inducing a group of regular smokers to stop smoking and to observe their cholesterol levels weekly over the ensuing months.

Itemized Annual Budget

Salaries

Director, Dr. Caroline Bedell Thomas	\$2,000
Fellow, part-time, Dr. Edmund A. Murphy	1,000
Chief Statistical Clerk	2,800
Assistant Statistical Clerk	2,400
Student Assistants	600

8,800

300

Expendable Supplies

Permanent Equipment

Other: costs of tabulation and publication, cholesterol determinations for smoking studies, etc.

300

\$ 9,400

1,830

1,410

\$ 12,840

Overhead (15%)

TOTAL

\$ 14,892

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6. Budget Plan:

Salaries
Expendable Supplies
Permanent Equipment
Overhead 15%
Other

\$8,800.
300.
00.
1,500.
300.
\$11,200.

Total

14100
108100

7. Anticipated Duration of Work:

two years.

8. Facilities and Staff Available:

There is ample work space in conjunction with our major project including a separate laboratory equipped with Starr-type ballistocardiograph and a double-channel Sanborn Viso-cardiette. Dr. Murphy, the part-time Fellow who has conducted the statistical studies of variability this year (see Progress Note Section II) is available and is well trained for the proposed studies. He has worked under Dr. John McKim Michael in England on the effect of hexamethonium in hypertensive patients. The entire project will be under my direct supervision, and will be assisted by the secretary and the physiological technician of the long-term project where required.

9. Additional Requirements:

Continuation of the advice and counsel of Miss Mary Burke, Statistician to the Tobacco Industry Research Committee.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The aims of the project outlined above are in harmony with those of Grant H-1891 (C3) entitled "Precursors of Hypertension and Coronary Artery Disease" awarded by the National Heart Institute. The funds from that source do not include most of the items covered by the budget given above. Where similar items exist in each of the two budgets, it is because the budget from Grant H-1891 (C3) is insufficient to meet the total expense of a given item, and the two budgets will be used in such a way that they supplement each other.

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Signature

/s/ Caroline Bedall Thomas
Director of Project

/s/ Thomas B. Turner, Dean, by
Business Officer of the Institution

Samuel P. Asper, Jr.
Associate Dean

(Y-TRE Grant #275)
Appl.

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COMPARISON OF SMOKERS AND NONSMOKERS

I. A PRELIMINARY REPORT ON THE ABILITY TO TASTE PHENYLTHIOUREA (P.T.C.)¹

CAROLINE BEDELL THOMAS AND BERNICE HIRSCHHORN COHEN²

Department of Medicine, The Johns Hopkins University School of Medicine

Received for publication October 5, 1959

Whether or not constitutional differences exist between smokers and non-smokers is a question which has recently become of medical importance. Because smokers have been found to have higher over-all death rates than nonsmokers, especially from cancer of the lung and coronary heart disease, it has been assumed by many that a causal relationship has been established between smoking and the diseases in question (1 to 7). However, the validity of this hypothesis has not been universally accepted (8, 9). Berkson, for example, has suggested several alternate explanations, one of which is that "persons who are nonsmokers or relatively mild smokers are constitutionally disposed to longevity, and therefore to generally lower death rates, and that the disposition of these individuals not to smoke is a reflection of this constitution" (9). There is already some evidence pointing toward psychologic, physiologic and metabolic differences between certain groups of smokers and nonsmokers (10 to 13). Demonstration of well established genetic differences would be even more conclusive, because such inborn traits are presumably stable and could not be the result of smoking. In a study of Johns Hopkins medical students, we have reported a higher incidence of hypertension and/or coronary disease among the parents of students who smoke than among the parents of their classmates who do not smoke (14). Twin studies have shown that the smoking habits of monozygotic twins, even when reared apart, are more alike than the smoking habits of dizygotic twins (15 to 17).

In order to explore the frequency of several genetic traits among groups of healthy smokers and nonsmokers, we have embarked upon studies of the characteristics of blood donors at the Johns Hopkins Hospital. The present report gives the preliminary findings in regard to the ability to taste phenylthiourea³ (P.T.C.) among various groups of healthy smokers and nonsmokers. Fox first detected great differences between people in regard to their taste sensitivity to P.T.C., a substance which tastes bitter to some individuals and is tasteless to others (18). Subsequent studies by Snyder and other investigators have shown that the inability to taste P.T.C. and closely related sub-

¹ This study was supported by The Tobacco Industry Research Committee.

² Postdoctoral Fellow of the National Heart Institute, United States Public Health Service.

³ Which was first known as phenylthiocarbamide.

stances is inherited through a single autosomal recessive gene (19 to 21). The test substance may be placed on the tongue in the form of crystals, as a drop of a solution of suitable strength, or in the form of filter paper impregnated with a standard solution of the test substance (18 to 24).

METHOD

Healthy persons accepted by the Blood Bank of the Johns Hopkins Hospital as suitable blood donors were given a standardized taste test and interviewed as to their smoking habits. The tests and interviews were carried out by one of us (B.H.C.) or by an assistant trained in interview techniques. Filter paper was soaked in a No. 2 solution of P.T.C. (650 mg. of crystalline phenylthiourea per liter of water) and dried. The taste test was performed by instructing the subject to place such a piece of P.T.C. impregnated paper on his tongue, moisten it well with saliva and leave it there a short time. He was then asked whether or not he tasted anything, and if so, what the taste was like. Subjects who stated that the taste was bitter were classified as "tasters" while those who said that they tasted nothing were rated as "nontasters". Those who could not be sure whether they tasted anything or not were scored as "uncertain", and those who said it had a taste such as sweet, sour, or pleasant were rated as "other". For the purposes of this study, these last two small groups will be combined under the term "atypical response".

The smoking habit questionnaire, which was administered and filled in by one of the staff members, was as follows:

A. I smoke _____	B. I used to smoke _____	C. I do not smoke _____
daily _____ occasionally _____	daily _____ occasionally _____	and never have _____
age begun _____	age begun _____	except a few times years ago _____
	age stopped _____	
total years of smoking _____	total years of smoking _____	
How many do you smoke per day?	How many did you smoke per day?	
Cigarettes: _____	Cigarettes: _____	
Cigars: _____	Cigars: _____	
Pipes: _____	Pipes: _____	
Do you inhale? yes/no	Did you inhale? yes/no	

The smoking habits thus recorded were then classified as follows:⁴

nonsmoker: one who has never smoked or, at most has smoked only a few times years ago.

occasional smoker: one who does not smoke daily, regardless of whether cigarettes, pipes or cigars are smoked.

cigarette smoker: (these subjects may smoke an occasional pipe or cigar).

a. light: 1-9 a day.

b. moderate: 10-19 a day.

c. heavy: 20 or more a day.

⁴ This is the summary of a smoking code used in our long-term study of the precursors of hypertension and coronary heart disease; it is also the one used by Heath and others (11, 12). We have also classified the smoking habits in a slightly different way according to the categories used in "Tobacco Smoking Patterns in the United States" in order to compare the distribution of smoking habits among our subjects with that found in a country-wide survey (25). However, only the classification given above is used in the present preliminary report.

pipe smoker:
cigar smoker:
pipe and cigar smoker: } (these subjects may smoke an occasional cigarette).
cigarette smoker combined with pipes and/or cigars (regular smoker).
former smoker: used to smoke regularly, nonsmoker now.

STUDY POPULATION

The subjects for the present report comprised all of the male donors coming to the Johns Hopkins Blood Bank on weekday afternoons from November 18, 1958 through May 29, 1959. There were 597 white male donors and 232 negro male donors. Women were also studied, but are excluded from the preliminary analysis because the numbers were so small (88 white women and 24 colored women).

RESULTS

The smoking habit patterns of the white and negro male donors, grouped according to ability to taste P.T.C., are shown in Table I. It will be seen that the frequency distribution of smoking habits is different for the taster and nontaster groups. Among both the white and negro men, a smaller proportion of tasters than nontasters were nonsmokers, while a larger proportion of tasters than nontasters were regular cigarette smokers. Dissimilarities in distribution between the white and negro groups appear when the various subdivisions of the regular cigarette smokers are considered. Among the white donors, 52.6 per cent of the tasters were heavy smokers in contrast to 29.7 per cent of the nontasters, while among the negro donors, a similar but less striking pattern of differences is observed among the light and moderate smokers. There were relatively few heavy smokers among the negroes and the absolute number was small, 64 in contrast to 258 heavy smokers among the white donors. It is of interest that 13 of the 17 atypical taste responses given by the 829 white and negro men combined occurred among the 322 heavy smokers and that no atypical taste responses were observed among the 124 nonsmokers.

We have compared smokers and nonsmokers as to their ability to taste P.T.C., grouping the various smoking habit categories in several ways. In Table II, nonsmokers are compared with heavy cigarette smokers, all other smokers (including occasional smokers) and former smokers, omitting those who gave atypical taste responses. In both white and negro donors, the differences in ability to taste P.T.C. found among these various smoking habit categories were statistically significant. The relatively small number of negro male donors precluded more detailed analysis of that group.

When the white male donors were grouped on the basis of cigarette smoking habits (omitting the pipe, cigar and mixed smoking habit categories as well

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TABLE I

Smoking Patterns of White and Negro Male Donors Grouped According to Ability to Taste P.T.C.

	Ability to Taste PTC	Total Donors		Never Smoked		Smoked Occasionally		Regular Smoker																Former Smoker	
								Cigarettes only								Pipes only		Cigars only		Cigars and pipes		Cigarettes pipes and/or cigars			
								Total		1-10		11-19		20 or more											
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%		
White male donors	Taster	323	100	41	12.7	14	4.3	221	68.4	18	5.6	33	10.2	170	52.6	10	3.1	7	2.2	3	0.9	6	1.9	21	6.5
	Nontaster	259	100	55	21.2	18	6.9	132	51.0	24	9.3	31	12.0	77	29.7	5	1.9	10	3.9	6	2.3	3	1.2	30	11.6
	Atypical	15	100	0	0.0	1	6.7	14	93.3	1	6.7	2	13.3	11	73.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Total	597	100	96	16.1	33	5.5	367	61.5	43	7.2	66	11.1	258	43.2	15	2.5	17	2.8	9	1.5	9	1.5	51	8.5
Negro male donors	Taster	124	100	9	7.3	16	12.9	85	68.5	36	29.0	20	16.1	29	23.4	1	0.8	4	3.2	3	2.4	2	1.6	4	3.2
	Nontaster	106	100	19	17.9	12	11.3	67	63.2	26	24.5	8	7.5	33	31.1	1	0.9	3	2.8	0	0.0	0	0.0	4	3.8
	Atypical	2	100	0	0.0	0	0.0	2	100.0	0	0.0	0	0.0	2	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Total	232	100	28	12.1	28	12.1	154	66.4	62	26.7	28	12.1	64	27.6	2	0.9	7	3.0	3	1.3	2	0.9	8	3.4

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TABLE II
Ability to Taste P.T.C. According to Smoking Habits Among 582 White Male Donors¹

	Tasters <i>N</i> = 323		Nontasters <i>N</i> = 259	
	Observed	Expected	Observed	Expected
Nonsmokers	41	53.3	55	42.7
Heavy cigarette smokers ²	170	137.1	77	109.9
All other smokers ³	91	104.3	97	83.7
Former smokers	21	28.3	30	22.7

$$X^2 = 32.2, n = 3, p < .001$$

Ability to Taste P.T.C. According to Smoking Habits Among 230 Negro Male Donors⁴

	Tasters <i>N</i> = 124		Nontasters <i>N</i> = 106	
	Observed	Expected	Observed	Expected
Nonsmokers	9	15.1	19	12.9
Heavy cigarette smokers ²	29	33.4	33	28.6
All other smokers ³	82	71.1	50	60.9
Former smokers	4	4.3	4	3.7

$$X^2 = 10.3, n = 3, .02 > p > .01$$

¹ 15 white donors who gave atypical responses to the taste test were excluded from this analysis.

² Those who smoke 20 or more cigarettes a day.

³ Includes occasional smokers, light and moderate cigarette smokers, pipe smokers and cigar smokers in any combination.

⁴ 2 negro donors who gave atypical responses to the taste test were excluded from this analysis.

as those with atypical taste responses), highly significant statistical differences were found:

1. Comparison of smoking categories of 10 cigarettes or less a day versus more than 10 cigarettes a day (nonsmokers, occasional and light cigarette smokers versus moderate and heavy cigarette smokers): $X^2 = 7.13$ $n = 1$ $0.01 > p > 0.001$.
2. Comparison of all five cigarette smoking categories (nonsmokers, occasional smokers, light smokers, moderate smokers and heavy cigarette smokers): $X^2 = 28.69$ $n = 4$ $p < 0.001$.

Regardless of how the donors are grouped, a positive association between heavier cigarette smoking and the ability to taste P.T.C. is observed.

The proportion of tasters in each smoking category of white donors is shown in Figure 1.⁵ This graph clearly shows that nonsmokers, former smokers,

⁵ Similar analysis of negro donors is deferred until more subjects have been studied, since at present there are very small numbers in many of the smoking categories.

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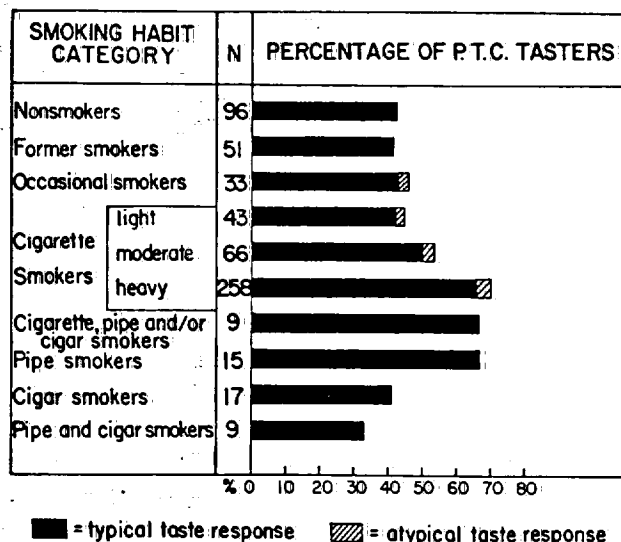


FIG. 1. Percentage of P.T.C. tasters among 597 white male donors classified according to current smoking habits. *N* = number of donors tested. Light cigarette smokers = 1 to 10 a day. Moderate cigarette smokers = 11 to 19 a day. Heavy cigarette smokers = 20 or more a day.

occasional smokers and light smokers are similar in regard to having a relatively low proportion of tasters. Heavy cigarette smokers, regular pipe smokers and mixed smokers also appear to resemble each other in exhibiting a higher proportion of tasters while moderate cigarette smokers occupy an intermediate position between light and heavy cigarette smokers.

We have examined the relationship of age to the proportion of tasters in each smoking group among white male donors; a summary of these findings is given in Table III.⁶ In general, it appears that there is no systematic alteration in the proportion of tasters from decade to decade where the numbers of subjects are sufficiently large. In every group except the oldest (which was much the smallest) the heavy cigarette smokers show the greatest percentage of tasters. Moreover, as shown in Table III, the percentage of tasters for each smoking category was not altered when age adjusted rates were used.

We have compared the results for the nine test periods in which different batches of P.T.C. test paper were used. No important differences were found attributable either to the test paper or to the season of the year. When the findings of the two interviewers were compared, slight differences were observed in the smoker-taster distribution in white male donors, but these were not statistically significant. Both interviewers found more tasters among the heavy cigarette smokers than among the nonsmokers.

⁶ The 15 white male donors who gave atypical taste responses were not included in this analysis.

TABLE III
Percentage of Tasters Among Donors Classified by Age and Smoking Habits

Age	Nonsmokers		Occasional Smokers		Heavy Cigarette Smokers ¹		Others Smokers ²		Former Smokers		Total: All Smoking Categories	
	N	% Tasters	N	% Tasters	N	% Tasters	N	% Tasters	N	% Tasters	N	% Tasters
White male donors												
Under 30.....	42	40.5	13	46.2	84	76.2	51	39.2	11	36.4	201	55.2
30-39.....	35	51.4	10	50.0	111	62.2	59	50.8	16	43.8	231	55.8
40-49.....	9	11.1	7	42.9	45	75.6	31	61.3	15	46.7	107	59.8
50 or over.....	10	50.0	2	0.0	7	42.9	15	53.3	9	33.3	43	44.2
Total: all ages.....	96	42.7	32	43.8	247	68.8	156	49.4	51	41.2	582	55.5
Age adjusted rates ³		40.0		43.9		68.2		49.0		40.8		54.7
Negro male donors												
Under 30.....	17	35.3	18	50.0	24	62.5	50	72.0	2	100.0	111	61.3
30-39.....	8	12.5	8	75.0	20	35.0	37	51.4	3	0.0	76	43.4
40-49.....	3	66.7	2	50.0	16	43.8	14	64.3	1	0.0	36	52.8
50 or over.....	0	0.0	0	0.0	2	0.0	3	66.7	2	100.0	7	57.1
Total: All ages.....	28	32.1	28	57.1	62	46.8	104	63.5	8	50.0	230	53.9
Age adjusted rates ³		31.0		57.3		47.8		63.4		50.0		54.0

¹ Heavy cigarette smokers = those who smoke 20 or more cigarettes a day.

² "Other smokers" includes all categories of regular smokers except heavy cigarette smokers.

³ The standard populations used for the age adjusted rates in the various smoking categories comprised 765 white male donors and 289 negro male donors studied at the Johns Hopkins Blood Bank between October 14, 1958 and May 29, 1959.

DISCUSSION

In this preliminary study, filter paper impregnated with P.T.C. was used as a rapid screening technique to ascertain whether smokers differ from nonsmokers in their ability to taste the test substance. In studies using solutions of P.T.C., the ability to taste has been shown to vary with the concentration of the solution (21, 24). Accordingly, the proportion of "tasters" to "nontasters" found in our studies, in which filter paper was used in a single test, cannot be directly compared with the proportions reported in studies of sensory thresholds carried out by the sorting technique of Harris and Kalmus or in investigations using still different methods (18 to 21, 26 to 28). Nevertheless, a standardized procedure was employed in the present study, so that the finding of significant differences between nonsmokers and smokers in ability to taste P.T.C. is of considerable interest. The fact that, among white male donors, the highest proportion of tasters occurred among heavy cigarette smokers and the lowest proportions among nonsmokers and former smokers appears to be clear cut and unrelated to age.

The apparent difference in taste response between white and negro smokers

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needs clarification through further studies involving larger numbers of negro smokers. Racial differences in tasting ability between white and negro groups in the United States have been reported (22, 29 to 32). If our preliminary findings are confirmed, the relative importance of constitutional differences and of socio-economic factors affecting the smoking habits of the two racial groups must be evaluated.

We cannot explain the reason for the observed differences between smokers and nonsmokers at the present time. The possibility that smoking directly affects the acuity of taste in respect to P.T.C. must not be overlooked. On the other hand, it is possible that inborn differences involved in the ability to taste P.T.C. may be part of a broad spectrum of genetically determined biochemical differences which both predispose individuals to their particular smoking habits and influence their susceptibility to certain diseases. Several clinical studies have been published indicating that deviations from the expected proportion of tasters to nontasters may be found in the presence of certain thyroid disorders, diabetes and tuberculosis (32 to 35). The possible interaction of many factors must be considered in evaluating the differential in specific and over-all mortality rates between smokers and nonsmokers.

SUMMARY

1. Taste tests, using filter paper impregnated with P.T.C., have been carried out on 597 white and 232 negro male donors at the Johns Hopkins Hospital Blood Bank. Each donor was interviewed as to his smoking habits.
2. Among both white and negro donors, significant differences in ability to taste P.T.C. were found between smokers and nonsmokers.
3. Among white male donors, heavy cigarette smokers showed a strikingly higher proportion of tasters than did nonsmokers (65.9 per cent versus 42.7 per cent). Former smokers, occasional smokers and light smokers closely resembled the nonsmokers as to proportion of tasters.
4. The significant findings were unrelated to age.

We should like to express our appreciation to Dr. Julius R. Krevans, Director of the Johns Hopkins Hospital Blood Bank, and his staff for their cooperation and assistance.

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PROGRESS NOTE

TOBACCO INDUSTRY RESEARCH COMMITTEE GRANT

I. PUBLICATIONS

A. Supported in part by TIRC grants.

1. Thomas, C.B. and Others: The Precursors of Essential Hypertension and Coronary Artery Disease: Collected Papers. Vol. I, 1948-1959.
2. Thomas, C.B. and Cohen, B.H.: Comparison of Smokers and Nonsmokers: I. A Preliminary Report on the Ability to Taste Phenylthiourea (P.T.C.). Bull. Johns Hopkins Hosp. 106:205, 1960.
3. Thomas, C.B.: Characteristics of Smokers Compared with Nonsmokers in a Population of Healthy Young Adults, including Observations on Family History, Blood Pressure, Heart Rate, Body Weight, Cholesterol and Certain Psychologic Traits. To be published in the Ann. of Int. Med.
4. Thomas, C.B. and Murphy, E.A.: Circulatory Responses to Smoking in Healthy Young Men. To be published by the New York Academy of Sciences in a monograph.
5. Thomas, C.B. and Murphy, E.A.: The Circulatory Response to Smoking: The Effect of Small Doses of Hexamethonium and of Mephentermine on the Pattern of Response. Submitted for publication.

B. Other.

1. Thomas, C.B. and Garn, S.M.: Degree of Obesity and Serum Cholesterol Level. Science, 131:42, 1960.

C. Ready for publication before Sept. 1, 1960.

1. Cohen, B.H. and Thomas, C.B.: Comparison of Smokers and Nonsmokers: II. ABO and Rh Blood Groups, with Further Observations on the Ability to Taste P.T.C.
2. Jones, L.W. and Thomas, C.B.: Studies on Figure Drawings: A Review of the Literature (1948-1959).

II. STUDIES IN PROGRESS

A. Genetic Differences between Smokers and Nonsmokers.

The following information was collected on 1620 healthy donors at the Johns Hopkins Hospital Blood Bank between October 14, 1958 and October 30, 1959:

1. Smoking habits of donors
2. Ability to taste P.T.C. (phenylthiourea, phenylthiocarbamide)
3. ABO and Rh blood groups
4. Cholesterol level

5. Smoking habits of parents
6. Medical history of parents as to high blood pressure, stroke, heart attack, other heart disease, cancer of lung and other cancer
7. Cause of death of parents

The collection period was terminated after 1009 white male donors had been registered. Dr. B.H. Cohen and the Director are now analyzing and publishing the results in a series of papers entitled "Comparison of Smokers and Non-smokers." The first paper, subtitled "I. A Preliminary Report on the Ability to Taste Phenylthiourea (P.T.C.)" appeared in the Bull. Johns Hopkins Hosp. 106:205, Apr., 1960 (attachment 3). The second paper, subtitled, "II. ABO and Rh Blood Groups, with Further Observations on the Ability to Taste P.T.C." is partially complete (attachment 6). Two more papers are contemplated, one dealing with the cholesterol levels of smokers vs. nonsmokers, the other with the smoking habits, causes of disability and of death of the parents of smokers vs. nonsmokers.

B. Psychological Differences between Smokers and Nonsmokers as Shown by Comparison of Figure Drawings: Draw-A-Person Test.

In the long-term "Precursors of Hypertension and Coronary Heart Disease" study on Johns Hopkins medical students, we have collected a vast amount of psychological data for correlation with genetic, physiologic and metabolic studies. The data include:

1. Questionnaires concerned with a detailed habit survey, interfamily attitudes and relationships and other personal data on over 1000 subjects (classes of 1948-1963).
2. Rorschach Tests on over 1000 subjects (classes of 1948-1963).
3. Figure Drawings (Draw-A-Person-Test) on 787 subjects (classes of 1952-1963).
4. Strong Vocational Interest Tests on 391 subjects (classes of 1958-1963).

Since the smoking habits of our subjects have been obtained on all classes, we have a unique opportunity of studying the personality characteristics of smokers compared with nonsmokers as they are revealed by projective and non-projective testing as well as by questionnaire.

Using interview and questionnaire methods, Heath and Lilienfeld have reported a variety of psychological differences between smokers and nonsmokers (1,2). In a preliminary analysis of the findings on our "habits of nervous

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tension" questionnaire, the occurrence of anger and urge to eat under stress was significantly greater among smokers than nonsmokers. (attachment 4). Nonsmokers more frequently reported decreased activity under stress than did smokers. However, when the Rorschach responses of smokers and nonsmokers were compared, no significant differences between the groups were found in respect to productivity, constriction, proportion of whole, detailed and very detailed responses or distribution of color responses. The only significant difference thus far identified was that nonsmokers more often gave a high number of white space responses than smokers. Many variables in the Rorschach test have not yet been examined.

Since September, 1958, we have studied the figure drawing material from several points of view. First, two pilot studies were carried out:

1. With the collaboration of Dr. Irvin Greenberg, clinical psychologist, almost 20 characteristics of the figure drawings of heavy cigarette smokers and nonsmokers were compared in a blind study. After excluding women and the foreign born, four groups of figure drawings were randomly selected, two consisting of the drawings of 24 heavy cigarette smokers each and two of the drawings of 24 nonsmokers each. The psychologist was given two unidentified sets of drawings; he was told that each set was homogeneous in regard to the subject's smoking habits, but was not told which group smoked. The variables studied in this fashion included:

- | | |
|--|---|
| a. Figure drawn first - man or woman | j. Figure smoking or not smoking |
| b. Figure size; difference in size of male and female figure | k. Amount of detail in drawing |
| c. Position of figures | l. Presence or absence of buttons, pockets, neckties, belts |
| d. Head and body concordant or discordant | m. Amount of hair - much, medium, little or none |
| e. Differences in general position and arm position of male vs. female figures | n. Mouth emphasis |
| f. Body parts missing; hands hidden, hands missing, ears missing | o. Mouth open or closed |
| g. Presence or absence of movement | p. Mouth shape |
| h. Figures nude or clothed | q. Breast emphasis |
| i. Presence or absence of extensions (knife, hat, gun, etc.) | r. Quality of lines: light vs. dark; sharp vs. shaded or broken |
| | s. "Global" characterization of drawings: primitive, childish, medium, adult or idiosyncratic |

If differences were found between the first two groups, he examined the second two unidentified sets of drawings to see if the same differences were again found. In several instances significant differences were found in comparing the first sets or the second sets, but consistent significant differences were not found in both sets. Although there were some suggestive findings, publication of these results was postponed until further studies could be carried out.

2. As part of the "Precursors" study, Dr. E.W. Slockbower, clinical psychologist, has written interpretations of the figure drawings of 787 students without knowledge of their smoking habits. The interpretations were examined blind by two nonpsychologists for differences in the reported frequency of selected personality characteristics. The interpretations for 96 heavy cigarette smokers and 96 nonsmokers were randomly selected and the groups of 96 were divided into four subgroups of 24 subjects each. The characteristics and their frequencies are given in Table A. (attachment 7). It will be seen that smokers more often showed obsessive-compulsive trends, feelings of inadequacy, impulsiveness, oversensitiveness, heterosexual and homosexual problems and immaturity, while nonsmokers exhibited shyness, passivity and identification problems. There was great similarity between smokers and nonsmokers in regard to the frequency of aggressiveness, orality, fear of castration and masturbatory guilt. Before making tests for statistical significance, however, it seems wise to further sharpen our definitions of terms and reproducibility of scoring. Publication is therefore temporarily postponed.

Next, a preliminary classification of figure drawings was made and scoring of 787 sets of figure drawings suitable for IEM analysis was undertaken by two nonpsychologists. It was found that whereas scoring was highly reproducible between observers for some items (e.g. sex of figure drawn first; figure nude or clothed) it was less reproducible in respect to other items (e.g. breast emphasis) and difficult to reproduce in regard to such items as quality of line or "global" impression.

At this point two things became clear: we needed an expert in psychological testing and statistics to collaborate with us, and it was important to review the literature on figure drawings to determine how others had handled the problems of validity and reliability.

We were most fortunate in being able to add Dr. Leona Wise Jones to our staff on a part-time basis on Dec. 1, 1959. As one who has taught in the field of psychological testing (see biographical note under 8. Facilities and Staff Available) she brings the needed critical judgment to our project. The Draw-A-Person Test has been in use as a clinical tool for a little over 10 years. Dr. Jones studied practically all of the papers pertaining to that test, and found that there is no comprehensive review of the literature; Swenson's "Empirical Evaluation of Human Figure Drawings" is the nearest thing to it, but the approach of that excellent paper was somewhat different from the one we are interested in (5). Accordingly, Dr. Jones has completed a 53 page first draft

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of a paper entitled "Studies on Figure Drawings: A Review of the Literature (1948-1959)" and is now revising it. The outline of the paper is as follows:

- I. Introduction
- II. Concept
- III. Aspects of Research
 - A. Differentiation
 - B. Methodology
 1. Judges
 2. Scoring Scales
 3. Procedures
 - C. Validity and Reliability
 - D. Interpretations

We are beginning to refine our own methods of classification, particularly in respect to reliability of scoring. In doing this, we shall make use of information derived from a number of sources. In particular, we are using Swenson's scale for rating sexual differentiation on the Draw-A-Person Test (4,5). Two projected papers based on our figure drawing material are:

- I. The Draw-A-Person Test: Comparison of Smokers and Nonsmokers in a Population of Medical Students, with Especial Reference to Sexual Differentiation and Identification.
- II. The Relationship of Body Type to Self-Image as Revealed by Figure Drawings.

These studies should prove of particular interest in the light of Seltzer's recent work on constitutional types (6). He found statistically significant differences in the strength of the masculine component among groups of men with different smoking habits. Weakness of the masculine component was significantly more frequent in smokers than nonsmokers, and most frequent among heavier smokers. Dr. Seltzer now has an independent TIRC grant to carry out similar studies on the Johns Hopkins medical students, who are also our subjects. He will use portions of our data, particularly concerning smoking habits, and in return will add his data to ours. This circumstance gives us special incentive to explore the psychological sex orientation of the students for purposes of seeking interdisciplinary correlations with his constitutional findings.

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3/28/60

ATT 2

PROGRESS NOTE FOR RESEARCH GRANT H-1891 (C5)

Dr. Caroline Bedell Thomas

The Johns Hopkins University
School of Medicine

Title: Precursors of Hypertension and Coronary Disease

June 1, 1959 through April 1, 1960

A. Professional personnel:

1. Dr. Caroline Bedell Thomas, Associate Professor of Medicine - 80% of time
2. Dr. Mildred Kendrick, Assistant, Div. Chr. Dis., J.H. School of Hygiene and Public Health - 100% of time 9/1/59-5/31/60

B. Collection of basic data on Johns Hopkins medical students has continued along lines previously described. The class of 1963 registered this year, is the sixteenth consecutive class studied, bringing the number of well-studied subjects to around 1000 at the time of their graduation in 1963.

C. Follow-up studies: the classes of 1950 and 1955 have just received their ten- and five-year questionnaires. Vigorous efforts are being made to secure 100% return.

D. Special investigations:

1. Effect of Vitamin B₁₂ on cholesterol level.
2. Seasonal variations in cholesterol level.
3. Comparison of figure drawings of smokers and nonsmokers (supported in part by the Tobacco Industry Research Committee).
4. Genetic factors among healthy smokers and nonsmokers (supported as in 3).

E. Analysis of data:

1. The general design of the project, the nature of the data obtained and plans for the future, including a) statistical analysis and publication of the basic findings and b) methods of long-term follow-up, are being reviewed in a series of conferences with a group of advisors from the Johns Hopkins School of Hygiene and Public Health: Professor Abraham M. Lilienfeld, Head of the Div. of Chr. Dis.; Dr. Raymond Seltzer, Assistant Professor in Epidemiology, and Dr. Earl L. Diamond, Assistant Professor of Public Health Administration and Assistant Professor of Biostatistics.
2. Topics thus far discussed include: a) study population, b) hypotheses of the study, c) reliability of the family history data, d) death certificates, e) follow-up information and f) construction of life tables for the parents of the medical students.

F. Other:

1. The 26 papers resulting from this study between 1948 and 1959 have been collected in a single paper-covered volume printed by the photo-offset method.
2. An anthropometric study has just been started in parallel to the present project under the direction of Dr. Carl Seltzer of Harvard sponsored by the Tobacco Industry Research Committee. Body measurements and constitutional photographs are to be obtained on 300 students in the Johns Hopkins School of Medicine and on as many graduates registered in our study as possible during the next year. Their data is to be turned over to us for use in the long-term study. We are to make available to them our data on smoking habits and certain other items.

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SUMMARY OF PAPER SUPPORTED BY THE TIRC TO BE PUBLISHED
IN THE BULLETIN OF THE JOHNS HOPKINS HOSPITAL, IN APRIL, 1960

Comparison of Smokers and Nonsmokers:

I. A Preliminary Report on the Ability to Taste Phenylthiourea (P.T.C.)

by

Caroline Bedell Thomas and Bernice Hirschhorn Cohen

SUMMARY

1. Taste tests, using filter paper impregnated with P.T.C., have been carried out on 597 white and 232 negro male donors at the Johns Hopkins Hospital Blood Bank. Each donor was interviewed as to his smoking habits.
2. Among both white and negro donors, significant differences in ability to taste P.T.C. were found between smokers and nonsmokers.
3. Among white male donors, heavy cigarette smokers showed a strikingly higher proportion of tasters than did nonsmokers (65.9% versus 42.7%). Former smokers, occasional smokers and light smokers closely resembled the nonsmokers as to proportion of tasters.
4. The significant findings were unrelated to age.

Caroline Bedell Thomas, Assistant Professor

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ABSTRACT OF PAPER SUPPORTED BY THE TIRC TO BE
PUBLISHED IN THE ANNALS OF INTERNAL MEDICINE

Characteristics of Smokers Compared with Nonsmokers in a Population of Healthy Young Adults, including observations on family history, blood pressure, heart rate, body weight, cholesterol and certain psychologic traits.

Caroline Bedell Thomas

The smoking habits of ten successive classes of Johns Hopkins medical students have been studied in relationship to certain genetic, physiologic, metabolic and psychologic characteristics. Smokers were significantly different from nonsmokers in a number of ways. As a group, smokers: 1) more often gave a history of parental hypertension, 2) had a higher mean heart rate and pulse pressure, 3) had higher cholesterol levels, 4) had a larger proportion of heavy individuals and 5) more often reported reactions of anger and an increased urge to eat when under stress. Nonsmokers: 1) more often gave a history that both parents were free from hypertension and coronary artery disease, 2) had a higher mean diastolic pressure, 3) more often reported decreased activity under stress and 4) more often gave a high number of white space responses in the Rorschach test.

A parallelism existed between the presence or absence of factors thought to indicate high susceptibility to hypertension and/or coronary disease, on the one hand, and the presence or absence of the habit of smoking on the other. The highest proportion of smokers was found in the most susceptible group, Group I, and the lowest proportion of smokers was noted in the least susceptible group, Group IV. Groups II and III were intermediate in both respects.

Broad areas were found where no significant differences appeared between the two groups. The characteristics of smokers and nonsmokers were similar in regard to 1) the responses of blood pressure and heart rate to the cold pressor test, the double Master exercise test and the ballistocardiographic smoking test, 2) academic excellence and 3) most of the Rorschach variables examined, including productivity, concentration, proportion of whole, detailed and very detailed responses, and distribution of color responses.

It cannot be determined from the present data whether individual characteristics which are more often found among smokers than nonsmokers represent constitutional differences or are due to the effects of smoking. The differences observed in the parental histories indicate that smokers and nonsmokers have a somewhat different heritage and suggest that at least some of the differences found in individual traits may be genetic in origin.

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ABSTRACT OF PAPER SUPPORTED BY THE TIRC PRESENTED TO THE NEW YORK
ACADEMY OF SCIENCES CONFERENCE ON MARCH 26, 1960; TO BE PUBLISHED IN A MONOGRAPH

Circulatory Responses to Smoking in Healthy Young Men

Caroline Bedell Thomas and Edmund A. Murphy

The circulatory response to cigarette smoking has been studied in healthy medical students by means of the ballistocardiographic smoking test. Control blood pressure, heart rate, and ballistocardiogram are obtained with the subject at rest on a Starr-type ballistocardiographic bed, after which the subject smokes two standard cigarettes in succession; measurements are repeated after each cigarette. Stroke volume, cardiac output and cardiac index are calculated from the ballistocardiogram.

Statistically significant mean changes in all measurements were found following one cigarette. The direction and degree of change after smoking varied greatly from subject to subject, resulting in strikingly different individual patterns. When subjects were grouped according to the presence or absence of parental hypertension or coronary disease, different patterns of response for the various groups were noted.

In reproducibility studies consisting of eight tests on each of six subjects the first test appeared to be an unbiased estimate of the mean. Tests performed with the subject in a fasting state resulted in lower control readings than those at other times of day, with a complementary increase in the response to smoking, so that the final figures after smoking were remarkably constant.

The response to the smoking test was compared with the response to the cold pressor test in 386 young men. In both tests, age and smoking habits affected the magnitude of the responses very little. Also, systolic pressure, diastolic pressure and heart rate showed approximately normal distribution so that they all appeared to be continuous variables; any dividing line to distinguish "hyperreactors" in either test seems purely arbitrary. Correlations between circulatory responses to the two tests were relatively low. Independent information is gained from the smoking test and from the cold pressor test. Accordingly, both tests contribute to the appraisal of individual circulatory reactivity.

A review of the records of 245 subjects as to ballistocardiographic form revealed that 7.4 per cent showed borderline or early abnormal changes after smoking. Less than 2.0 per cent showed deviations from normal form before smoking. A significantly greater proportion of subjects with form changes were overweight and had high cholesterol levels compared with subjects with normal ballistic form.

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ABSTRACT OF PAPER IN THE PROCESS OF COMPLETION SUPPORTED BY THE TIRC
 Comparison of Smokers and Nonsmokers: II. Further Observations on
 the Ability to Taste P.T.C.; ABO and Rh Blood Groups.

by

Bernice Hirschhorn Cohen and Caroline Bedell Thomas

The current report involves the same method and study population described in the previous one, but gives the findings in a second series of donors. The trends in the second series are not only similar in direction but even more marked than those of the published group (Series I) in regard to the smoker-taster differences among white male donors. In addition, a similar but less marked trend was observed among negro male donors who had displayed some irregularities in the first series.

Among white male tasters of Series II, 73.7% were cigarette smokers and 12.0% never smoked, whereas among nontasters only 33.7% were regular cigarette smokers and 37.0% had never smoked (Table I). Among negro males of the new series, tasters included 72.3% cigarette smokers and 14.9% nonsmokers, whereas nontasters included 66.7% cigarette smokers and 17.6% who had never smoked. Among white male donors, 56.4% of tasters and 21.7% of nontasters are classified as "heavy cigarette smokers" (20 or more a day), while among negro male donors, 46.2% of tasters and 21.6% of nontasters are heavy cigarette smokers. That these differences in smoking habits and taster ability are not a function of age is indicated by a comparison of age adjusted rates (Table 2).

The ABO and Rh blood types of the subjects were also examined. Table 3 shows the distribution of A, B, and O and AB white male donors of pooled Series I and II by smoking category. The distribution of ABO blood groups in the total white male donors of Series I and II (1005 subjects) agreed closely with the frequencies reported for U.S. whites by Glass and Li based on samples from New York City and North Carolina, and are in all likelihood representative of the Baltimore area. On the other hand, the distribution of total negro male donors (365 subjects) was significantly different from population expectancy with an excess of O and an overall deficiency of A individuals.

The overall proportion of Rh negative white donors (15.8%) did not differ appreciably from the 14.4% estimated for New York City whites, nor did the proportion of Rh negative negro donors (7.1%) differ from their expectancies of 7%.

When white males are divided into five smoking categories (nonsmokers, occasional smokers, regular smokers, other smokers and former smokers) no significant differences in ABO distribution were found between the groups. However, comparison of total cigarette smokers and the pooled group of occasional smokers and nonsmokers attained the 2% level of significance ($\chi^2_3 = 10.6$, $.02 > p > .01$) and this difference was also marked when heavy cigarette smokers, occasional and nonsmokers were grouped separately and compared ($\chi^2_6 = 14.7$, $.05 > p > .02$). Examination of Rh groups showed that occasional smokers had a significantly higher frequency of Rh negatives (29.4%) than heavy, other or former smokers ($\chi^2_4 = 11.1$, $.05 > p > .02$). Negro male donors also showed some differences in ABO distribution among nonsmokers, occasional smokers and heavy smokers ($\chi^2_6 = 9.2$, $.05 > p > .02$), but no real deviations in the proportion of Rh negatives.

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TABLE A

PROVISIONAL SUMMARY OF SELECTED PERSONALITY TRAITS OF RANDOMLY CHOSEN GROUPS OF NINETY-SIX
SMOKERS AND NINETY-SIX NONSMOKERS ACCORDING TO FIGURE DRAWING INTERPRETATION

	Smokers						Nonsmokers					
	F ₁	F ₂	Q ₁	Q ₂	Total	Mean	A ₁	A ₂	J ₁	J ₂	Total	Mean
N:	24	24	24	24	96		24	24	24	24	96	
Aggression-hostility	18	19	16	17	70	17.50	19	19	17	15	70	17.50
Obsessive-compulsive trends	9	8	4	6	27	6.75	7	4	4	3	18	4.50
Inadequacy, inferiority, insecurity	17	17	11	11	56	14.00	13	14	13	11	51	12.75
Impulsiveness	1	3	1	2	7	1.75	2	2	1	0	5	1.25
Shyness, inhibition, withdrawnness	6	7	5	5	23	5.75	6	12	7	6	31	7.75
Oversensitiveness, paranoid trends, overawareness	11	7	6	6	30	7.50	7	6	2	6	21	5.25
Passivity	3	8	4	9	38	9.50	8	10	6	14	38	9.50
Orality	18	23	22	22	84	21.00	21	24	19	21	85	21.25
Heterosexual problems	3	5	2	9	19	4.75	2	3	4	5	14	3.50
Homosexual problems	7	1	4	6	18	4.50	1	3	6	4	14	3.50
Masturbatory guilt	1	3	3	2	9	2.25	2	4	4	0	10	2.50
Identification problems	3	4	3	2	12	3.00	3	6	3	6	18	4.50
Immaturity	14	9	11	9	43	10.75	8	12	8	10	38	9.50

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TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

#127

Application For Research Grant

Date: March 9, 1956

1. Name of Investigator: 1) R. W. Tiecke
2) J. C. Calandra
2. Title: 1) Associate Professor of Pathology
2) Professor of Pathology
3. Institution
& Address: Northwestern University Dental School
Chicago 11, Illinois
4. Project or Subject: The Role of Tobacco in Oral Cancer

5. Detailed Plan of Procedure (Use reverse side if additional space is needed).

- A. Specific Aims. The aim of the project is to determine whether the use of tobacco acts as a contributing factor in the cause of oral cancer.
- B. Method of Procedure. This will be done by the application of tobacco tars on the oral mucosa of rabbits. The clinical normal appearing oral mucosa of these animals will be examined under the microscope first to serve as a guide for the normal. These materials will be applied to the oral mucosa in varying quantities and for varying periods of time. The animals will be sacrificed at intervals to be determined later and the treated areas examined microscopically. The length of exposure to the tobacco product, location, description of any lesion that arises and microscopic findings will be tabulated.
- C. Significance of this Research. Very little actual work has been done in this field. There are many papers which cite statistics in an attempt to prove that oral cancer is caused by the use of tobacco. However, nothing has been done to prove or disprove this in the laboratory. Evidence pointing one way or another would be a valuable contribution and would point to the interest the dentist too has in the cancer program. This area of the body in relation to cancer is all too often neglected or glossed over and included with cancer of the gastro-intestinal tract or respiratory tract, even though it constitutes from 10 - 15% of all cancer in the human being.

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6. Budget Plan:

1) 1 Soc. Security	\$ 102.00
2) Technician - full time	3,600.00
3) Animal caretaker - half time	1,500.00
Salaries	\$5,202.00
Expendable Supplies	1,725.00
Permanent Equipment	528.00
Overhead	1,118.00
Other	
Total	\$8,573.00

7. Anticipated Duration of Work: Two years -- The above total is for one year.
Total for two years would be \$17,146.00

8. Facilities and Staff Available:

- 1) oral pathologist
- 2) general pathologist
- 3) oral surgeon
- 4) two graduate students
- 5) bacteriological laboratory and equipment
- 6) histopathology laboratory and equipment

9. Additional Requirements:

10. Additional Information (including relation of work to other projects and other sources of supply):

- 1) Work is being done on Papanicolaou Technique in relation to oral cancer.
- 2) Expect to start work in Tissue Culture in the immediate future.
- 3) An adequate number of patients are available for this project from Cook County Hospital, VA hospitals in the area and from our own clinics.
- 4) After this work is completed, additional work should be done in relation to heat and cancer of the mouth.

Signature s/ Richard W. Tiecke
Director of Project

s/ A. T. Schmehling
Business Officer of the Institution

(Asst. Business Manager)

1003537186

CROSS REFERENCE SHEET

Name or Subject

JIRC

Regarding

Re Grant

SEE

L. H. Benham

1003537187

ck

92

Application For Research Grant

Date: April 28, 1955

1. Name of Investigator: Janet Travell, M.D.
2. Title: Associate Professor of Clinical Pharmacology
3. Institution & Address: Cornell University Medical College
1300 York Avenue
New York 21, New York
4. Project or Subject: Electrocardiographic Effects of Nicotine in the Rabbit with Experimental Coronary Atherosclerosis.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Using a special test animal, we propose to determine whether nicotine injected intravenously produces electrocardiographic effects characteristic of ~~myocardial ischemia~~ coronary insufficiency.

Coronary atherosclerosis will be produced in male rabbits by feeding a high cholesterol diet. Controls will be fed the same basic stock diet without cholesterol. In such cholesterol-fed rabbits, we have observed the development of occlusive coronary atherosclerosis in from 3 to 5 months, as indicated by ergonovine-induced electrocardiographic changes and pathologic study of the heart (Rinzler, S.H., Travell, J. and Karp, D.: Detection of Coronary Atherosclerosis in the Living Animal by the Ergonovine Stress Test. Science, in press 1955)

The ergonovine stress test will be used to detect the presence of coronary atherosclerosis in the living rabbit. The procedure is currently as follows: With the animal under nembutal ~~anesthesia~~ ~~anesthesia~~ anesthesia (15 to 20 mg. Kg.). two control electrocardiograms are taken with the Sanborn Twin-Beam Cardiograph, using Leads II and V₄ and paper speeds of 25 and 75 mm./sec. Ergonovine maleate (0.05 mg. Kg.) is injected intravenously, and Leads II and V₄ are repeated after 1, 3, 5, 10 and 15 to 20 minutes. ~~Positivity~~ Positivity of the test is indicated by S-T segment depression of 0.5 mm. or more. When ~~spontaneous~~ spontaneous S-T segment depression is present in the control electrocardiogram before ergonovine, positivity is indicated by further depression of this segment. The time-sequence of events suggests that these changes are the result of myocardial ischemia secondary to coronary vasoconstriction.

1003537188

Although nicotine increases the heart rate in many species, preliminary experiments indicate that in the rabbit suitable doses of nicotine do not produce a tachycardia. This is important since a drug-induced tachycardia may interfere with the interpretation of electrocardiographic changes in the S-T segment and T waves.

If a coronary vasoconstrictor action of nicotine is demonstrated, antagonistic and synergistic actions of nicotine with other coronary vasoconstrictor and vasodilator drugs, such as vasopressin and nitroglycerin will be investigated. It has been reported that nicotine may exert effects on the coronary circulation through stimulation of the posterior pituitary gland and release of vasopressor hormones. It is anticipated that during the course of this investigation, new data will be accumulated with reference to the action on the coronary circulation of such drugs as vasopressin and oxytocin.

1003537189

6. Budget Plan:

Includes Twin-beam Sanborn
Electrocardiograph & table \$1600

Salaries
Expendable Supplies
Permanent Equipment
Overhead
Other

(Full-time Research
Assistant and Part-time
Laboratory man

Pathology

Total

\$5,200.
800.
1,900
810.
200.
\$8,910.

7. Anticipated Duration of Work:

July 1, 1955 to June 30, 1956

8. Facilities and Staff Available:

The usual facilities of the Department of ~~Ex~~ Pharmacology.
Seymour H. Rinsler, M.D. and Dorothy Karp, Ph.D., will
be available for research assistance on this project.

9. Additional Requirements:

This part of the investigation should be completed in one
year. However, we expect to derive new ~~inter~~ leads that
may deserve further study.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The study concerning the ~~inter~~ detection of coronary atherosclerosis
in the living animal was carried out under a grant from the National
Heart Institute, National Institutes of Health, Public Health Services.
Continuing support for studies on the role of the skeletal muscles in cardiovascular
disease, in the amount of \$6,000 annually has been committed for two more
years.

Signature

/s/ James Travell, M.D.

/s/ Hugh Luckey, M.D.

Dean, Cornell University Medical College

1003537190

92 R1

Application For Research Grant /RENEWAL

Date:

April 12, 1956

1. Name of Investigator:

Janet Travell, M.D.

2. Title:

Associate Professor, Clinical Pharmacology

3. Institution

& Address:

Cornell University Medical College
1300 York Avenue
New York 21, N. Y.

4. Project or Subject:

Cardiac Effects of Nicotine in the Rabbit with Experimental
Coronary Atherosclerosis.

1003537191

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

The proposed program for next year is the same as this year's with respect to the electrocardiographic effects of intravenous nicotine on the atherosclerotic heart of the cholesterol-fed rabbit. To complete this part of the study as previously outlined, we need larger numbers of animals in the series and also further comparisons of nicotine with vasodilator and vasoconstrictor drugs.

To recapitulate, the plan is as follows: Coronary atherosclerosis will be produced in male rabbits (Dutch-belted breed) by feeding a 2 per cent cholesterol diet. Controls will be fed the same basic stock diet without cholesterol. In such cholesterol-fed rabbits, we have observed the development of occlusive coronary atherosclerosis in from 3 to 5 months, as shown by the appearance of ergonovine-induced electrocardiographic changes in serial tests and final pathologic study of the heart (Rinsler, S.H., Travell, J. and Karp, D.: Detection of Coronary Atherosclerosis in the Living Animal by the Ergonovine Stress Test, Science, 121:900, 1955; Rinsler, S.H., Travell, J., Karp, D. and Charleson, D.: Detection of Coronary Atherosclerosis in the Living Rabbit by the Ergonovine Stress Test, Am. J. Physiol., in press, 1956.) The ergonovine test will be used to determine when coronary atherosclerosis has developed, as described in the above reports.

So many questions have arisen concerning the dynamics of the circulation in the intact animal that we have this year enlarged the scope of our investigations to include perfusion of the coronary arteries of the isolated heart of both normal and cholesterol-fed rabbits. We were surprised at first to find that the diseased heart would continue to beat satisfactorily in the perfusion apparatus. The accompanying abstract (Karp, D., Penna, M., Rinzler, S.H. and Travell, J.: Effect of Ergonovine on the Heart, *J. Pharm. and Exper. Therap.* 116:34, 1956) presents our preliminary results of studies of this type with ergonovine. This approach, utilizing the isolated perfused heart, yields fundamental data on the precise mechanisms of action of drugs with respect to three separate cardiac tissues: (1) myocardium, (2) smooth muscle of the coronary arteries, and (3) intrinsic pacemaker system.

(Reprinted from The Journal of Pharmacology and Experimental Therapeutics
January, 1956)

Effects of ergonovine on the heart. Dorothy Karp, Marie Penna, Seymour H. Rinzler and Janet Travell. Dept. of Pharmacology, Cornell Univ. Med. College, N. Y.

Intravenous ergonovine causes S-T segment depression in patients with effort angina of coronary insufficiency and in rabbits with experimental coronary atherosclerosis and myocardial damage, but not in man or rabbit with normal coronary circulation (Rinzler, Travell and Karp; *Science*, 121: 900, 1955). In rabbits dosage was 0.05 mg./kg., about 10 times that used for ergonovine tests in patients. Experiments to determine mechanisms by which ergonovine alters the electrocardiogram were carried out first on cat papillary muscle. In concentrations up to 0.8 mg./l. ergonovine had no direct action on cardiac muscle in this species. The isolated rabbit heart was perfused with ergonovine (modified Langendorff procedure). Doses of 0.05-2 mg. were injected into the system close to heart; effects were observed for 5-15 min. In normal rabbits, ergonovine at lower concentrations caused slight reduction both in coronary flow and force of contraction, but in higher concentrations as a rule caused increase in coronary flow and further reduction in force. In the cholesterol-fed rabbit with a positive ergonovine test and coronary atherosclerosis seen on subsequent microscopic examination, similar perfusion of the heart with ergonovine caused profound brief reduction in coronary flow together with reduction in contractile force. In the same preparation, vasopressin caused a similar reduction in flow but with an increase in force; if coronary constriction was prolonged, diminution in force superseded. Thus, opposite effects of ergonovine on coronary flow may be seen in rabbit hearts with normal and impaired coronary circulation; direct depression of contractile force is of the same order in both. We conclude that specific electrocardiographic effects of ergonovine imply constriction of coronary arteries by the drug. (Supported by grants from the National Heart Institute, National Institutes of Health, Public Health Service, Grant H-493 and the Josiah Macy, Jr. Foundation.)

1003537192

6. Budget Plan:

Full-time Research Asst. & part-time lab. man	\$5,200.
Salaries	2,056
Expendable Supplies	450
Permanent Equipment	1,000
Overhead	1,930
Other Pathology, photography	364
5% retirement, 2% Social Sec. Total	TOTAL \$11,000

7. Anticipated Duration of Work:

Two years

8. Facilities and Staff Available:

The usual facilities of the Department of Pharmacology. Seymour H. Rinzler, M.D. and Dorothy Karp, Ph.D., will be available for research assistance on this project.

9. Additional Requirements:

July 1, 1957 - June 30, 1958
\$10,000. plus overhead

10. Additional Information (Including relation of work to other projects and other sources of supply):

The study concerning the detection of coronary atherosclerosis in the living animal was carried out under a grant from the National Heart Institute, National Institutes of Health, Public Health Service. Continuing support for studies on the role of the skeletal muscles in cardiovascular diseases, in the amount of \$6,000 annually has been committed for two more years.

Signature S/S Janet Travell, M.D.
Director of Project

S/S E. K. Taylor, Asst. Treasurer
Business Officer of the Institution

S/S E. Hugh Luckey, M.D., Dean

1003537193

CONFIDENTIAL

TIRC GRANT #92

Progress Report #2

Dr. Janet Travell
Cornell University Medical College

January 1, 1956 - July 1, 1956

"Electrocardiographic Effects of Nicotine in the Rabbit
with Experimental Coronary Atherosclerosis"

1. Methodology

1. Animal care

In the interim report, problems dealing with animal care were discussed. We had concluded that the Dutch belted rabbit on the cholesterol diet seemed to survive longer than the albino rabbit. This consideration is of paramount importance since it takes a long time for coronary atherosclerosis to develop. We noted, however, that in the first weeks on the experimental diet the animals are apt to develop a fatal infectious illness.

The following change was therefore made in procedure: On arrival from the farm, all animals are put on a stock diet for 10 days and given Sulmet (Lederle sulfamethylthiazole) intraperitoneally every other day for 3 injections, each 200 mg. total. At the end of 10 days, the animals are continued on the stock diet if in the control group, or shifted to the cholesterol diet. We still employ a cholesterol diet containing 2% cholesterol and 6% corn oil.

II. Results of Electrocardiographic Studies in the Intact Animal

1. Control Animals

In 27 tests done on different days on normal rabbits under Nembutal anesthesia (20 mg./Kg., i.v.), nicotine in doses of 0.1, 0.25, 0.5 and 1.0 mg./Kg. intravenously produced no change in the electrocardiogram which might be regarded as indicative of an effect on the coronary circulation, when observed for periods of 10 to 15 minutes after injection of the drug.

2. Cholesterol-fed Animals

Since the atherosclerotic rabbits with positive ergonovine tests were sick animals, and their survival for coronary perfusion was desired, the intravenous dose of nicotine in this group was reduced to 0.05 mg./Kg. This is still large in terms of the level of nicotine in the blood stream after smoking.

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In 9 cholesterol-fed animals with a positive ergonovine test (Table I), the intravenous injection of this dose of nicotine bitartrate (0.05 mg/Kg.) produced electrocardiographic changes clearly indicative of coronary insufficiency in one animal, namely, an S-T segment depression of 0.5 mm. (Table 1, M₆). Microscopic examination of the coronary arteries in 8 of these animals showed atherosclerosis; one section is not yet available for study.

The effect of nicotine on the rate of these atherosclerotic hearts is also indicated in Table 1. From the average control rate of 248 beats/min., the rate at the end of 10 minutes had dropped to 229 beats/min. On the average, a fall in rate began within one minute after nicotine injection. Two animals showed an initial acceleration of heart rate.

III. Results of Perfusion Studies of the Isolated Rabbit Heart.

The effect of nicotine bitartrate on the amplitude and rate of contraction and on the coronary flow in the isolated preparation of the atherosclerotic heart (following a positive ergonovine test) was studied in 10 hearts with a dose of 0.05 mg., and in 8 hearts with a dose of 0.1 mg. With the smaller dose of nicotine, the maximal change in amplitude expressed in per cent of the control level, was +24.5%; for heart rate, it was -5.8%; and for coronary flow it was -1.4%. With the larger dose of nicotine, similarly the average percentage change in amplitude from the control level was +53.6%; for heart rate, it was -2.6% and for coronary flow -19.5%. Thus, nicotine in a relatively large dose increases the amplitude of contraction, has little effect on heart rate, and may significantly reduce coronary flow. More observations are needed on the individual variability of this effect on coronary flow, which is not uniform.

Additional perfusion experiments will have to be done before it can be stated whether normal and atherosclerotic hearts react similarly to nicotine with respect to these different structures: myocardium, arterial musculature, and the pacemaker and conduction system.

1003537135

TABLE I

Effect of Intravenous Nicotine Bitartrate (0.05 mg./Kg.) on the Electrocardiogram in 9 Cholesterol-Fed Rabbits with Positive Ergonovine Tests (Nembutal Anesthesia).

Rabbit No.	Control Heart Rate	Heart rate-minutes after injection				E.C.G. changes after nicotine	Coronary Arteries
		1/4	1	5	10		
M5	248	144	130	172	180	T-wave (lead II) from upright to diphasic	Plaques
M6	300	300	300	284	264	S-T segment depressed 0.5 mm.	Foam cells
P1	210	212	209	205	201	T-wave (lead II) inverted; decreased amplitude of T-wave (V ₄) from 4 to 2 mm.	Plaques
Q2	170	240	175	170	162	None	Plaques
Q2	260	254	245	259	250	None	Plaques
Q7	245	300	285	275	250	None	Plaques
Q9	225	215	210	210	205	None	Plaques
M1	248	246	240	240	240	None	Plaques
U9	323	305	320	320	310	Decreased amplitude of R-wave in (V ₄)	-
Averages	248	247	235	237	229		

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TABLE 2

Effects of Nicotine (0.05 and 0.1 mg.) on the Perfused Atherosclerotic Rabbit Heart

No. of Hearts	AMPLITUDE OF CONTRACTION				HEART RATE				CORONARY FLOW			
	Ringer-Locke mm. % change		Nicotine mm. % change		Ringer-Locke beats/min. % change		Nicotine beats/min. % change		Ringer-Locke cc./min. % change		Nicotine cc./min. % change	
10	17.0	-1.1	7.0	/ 24.5	108	-0.9	<u>Nicotine 0.05 mg.</u> 105	-5.8	19.9	-4.7	14.4	-1.4
8	17.1	-1.7	7.1	/ 53.6	106	-1.6	<u>Nicotine 0.1 mg.</u> 119	/ 2.6	19.8	-3.9	14.0	-19.5

1003537197

NO CHARTS ATTACHED

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

RENEWAL
Application For Research Grant

#9282

July 1, 1957 - June 30, 1958

Date: April 11, 1957

1. Name of Investigator: Janet Travell, M.D.
2. Title: Associate Professor of Clinical Pharmacology.
3. Institution & Address: Cornell University Medical College,
1300 York Avenue
New York 21, New York
4. Project or Subject: Cardiac Effects of Nicotine in the Rabbit with Experimental
Coronary Atherosclerosis.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Briefly to summarize our definitive results to date:

In cholesterol-fed male rabbits, we have observed the development of occlusive coronary atherosclerosis usually in from 4 to 6 months, as shown by the appearance of ergonovine-induced electrocardiographic changes in serial tests and by final pathologic study of the heart. Every animal with a positive ergonovine test has shown coronary atherosclerosis at postmortem.

One-eighth of 16 ergonovine-positive rabbits, similarly tested with intravenous nicotine bitartrate, have shown acute electrocardiographic changes suggestive of constriction of the coronary arteries (S-T segment depression). No such changes were observed in 12 normal rabbits.

Perfusion of the coronary arteries of the isolated heart has been carried out on 16 atherosclerotic hearts of ergonovine-positive rabbits and on 14 normal rabbits. Data on the effects of nicotine in these experiments relate to coronary flow, amplitude of contraction and heart rate.

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The results (Travell, J., Karp, D. and Rinzler, S. H.: Nicotine Effects on Normal and Atherosclerotic Hearts, Federation Proc., p. 341, 1957) show that the immediate effect of nicotine is consistently a decrease in coronary flow. On the average, the degree of vaso-constriction for the larger doses used (0.05-0.1 mg.) appears to be greater for the normal than for the atherosclerotic heart; in no instance did an atherosclerotic heart show significant coronary vasodilation after nicotine. That the atherosclerotic coronary system can dilate at this time is shown by its vasodilator response to nitroglycerin. Effects of nicotine on heart rate and amplitude of contraction were qualitatively similar in normal and atherosclerotic hearts.

In the atherosclerotic isolated heart, nicotine appears to be a less potent coronary constricting agent than either ergonovine or ~~xxx~~ vasopressin. Preliminary experiments indicate that the effects of nor-epinephrine in the isolated heart are similar to those of nicotine.

Our chief objectives next year will be to:

- 1) Determine the stage of coronary atherosclerosis at which a change occurs in the reactivity of the coronary tree to ergonovine and nicotine.

For this, we will perfuse the heart of the ergonovine-negative cholesterol-fed rabbit, and relate the results to the pathologic changes seen in the coronary arteries after perfusion. At the termination of perfusion, on section of the heart satisfactory pathologic detail is obtained with respect to early intimal lipid deposits, later foam cells and plaques, and finally deterioration of the elastic membrane beneath the plaques. Pathologic definition of the myocardium is poor after perfusion.

- 2) Shorten the time required to produce experimental coronary atherosclerosis in the cholesterol-fed rabbit.

Measures will be tried such as anti-thyroid drugs (effective in the dog), cold stress (effective in the rat), or hormone administration. A larger supply of atherosclerotic hearts is needed for direct study in the perfusion apparatus and possibly also for the papillary muscle preparation. The latter would represent a new step in the pharmacology of the atherosclerotic heart.

- 3) Increase the sensitivity of the ergonovine test so as to detect coronary atherosclerosis earlier in its course.

Possibly this may be accomplished by modification of dosage, combination with some other vasoconstrictor drug, or by substituting another vasoconstrictor agent for ergonovine.

- 4) Elucidate mechanisms of action of nicotine and other agents on the atherosclerotic as compared with the normal heart.

The influence of autonomic blocking agents on the response to nicotine in normal and atherosclerotic hearts will be investigated.

Comparisons of the effects of some coronary dilator agents may also be informative.

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- 5) Extend the electrocardiographic study of nicotine effects to the isolated heart.

In view of the low incidence of acute electrocardiographic changes after nicotine (0% in normal, 12.5% in atherosclerotic rabbits), it does not seem worthwhile at the moment to accumulate further data on the electrocardiographic effects of nicotine in the intact animal.

We propose to extend this phase of the work to include electrograms of the isolated heart during perfusion.

The addendum presents in tabular and chart form some of the data derived from this investigation.

1003537200

6. Budget Plan:

Banborn Visoscope,
Model 169 A with
booster amplifier

Part-time research associate
(S.H. Rinaler, M.D.)
Full-time research assistant, Ph.D.
Part-time laboratory man

Salaries	\$9,100.
Expendable Supplies	3,000.
Permanent Equipment	570.
Overhead (15%)	1,470.
Other	1,393.
Pathology, photography	637.
5% retirement, 2% social security	16,170.00 TOTAL

7. Anticipated Duration of Work:

Two years

8. Facilities and Staff Available:

The usual facilities of the Department of Pharmacology.
Dr. Dorothy Karp will be replaced by a new Ph.D. assistant.
Dr. Seymour H. Rinaler will next year be on our payroll.

9. Additional Requirements:

July 1, 1958 - June 30, 1959
\$15,000. / overhead

10. Additional Information (Including relation of work to other projects and other sources of supply):

The grant from the National Heart Institute, National Institutes of Health, Public Health Service, for studies on cardiovascular pain will expire this year, on September 30, 1957.

Signature _____

E. J. Travell

Business Office of the Institution

1003537201

INITIAL STATE OF ISOLATED PERFUSED HEART OF RABBIT

Heart	Total Number	Coronary Flow	Heart Rate	Amplitude of Contraction
		ml./min.	beats/min.	mm.
Normal	32	15.7 (9-28)*	151 (98-200)	33.0 (7-79)
Athero- sclerotic	29	19.5 (8 - 32)	126 (36-196)	26.9 (6-52)
t-test		$p < 0.01$	$p < 0.01$	$p < 0.001$

*Figures in parentheses indicate range.

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1003537203



1003537204

V

Lung Physiology
Committee:
Dr. Cattell
Dr. Bing
Dr. Rienhoff

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street New York 17, N.Y.

#289

Application For Research Grant

Date: August 23, 1960

1. Name of Investigator: Enrique Valdivia, M.D.
2. Title: Assistant Professor of Pathology
3. Institution: The University of Wisconsin Medical School
& Address: 426 North Charter Street
Madison 6, Wisconsin
4. Project or Subject: Microscopic Observations of the Pulmonary Vessels "In Vivo."

Pulmonary vessels of dogs and guinea pigs will be observed microscopically in vivo. When the normal anatomic relations of the various vascular patterns are established they will be compared with those of animals subjected to hypoxia. Similar observations will be made to determine the effect of various vasomotor drugs upon the pulmonary vasculature.

5. Detailed Plan of Procedure:

The study of acclimatization to high altitude has been the primary interest of the applicant over a period of years. Most of the previous investigation has been done by exposing guinea pigs to experimental chronic hypoxia in low pressure chambers. Tissue alterations, rapid dilation and hypertrophy of the right heart have been observed in the experimental animals. Radiological and histological studies have demonstrated evidence of right heart failure and increase in size of the branches of the pulmonary artery. These findings, and the reported evidence of pulmonary artery hypertension in acute and chronic hypoxia, indicate that there must be alterations in the pulmonary vasculature. For this reason, it was decided to observe these vessels "in vivo", under controlled physiological conditions and under different experimental variables.

Our method of procedure has followed two lines of approach to the problem, the first is the observation of pulmonary vessels "in vivo" in dogs and guinea pigs; the second is to establish the anatomic relations of the structures being observed in the same animals. This anatomical study is being done with radiograms obtained after the injection of the bronchial tree, pulmonary artery and pulmonary veins with a radio-opaque medium. Additional information is also obtained from casts of the same structures made after the injection with vinylacetate. The extent of the injections and minute anatomical relations are also checked by histological examination.

The observations of the pulmonary vessels "in vivo" are made at magnifications of 50 X to 750 X, measurements and some enlargement is obtained from cinematographic records. The preparation requires general anesthesia, open thorax and immobilization of the lung. Determinations of pulmonary artery pressure, femoral artery pressure, breathing rhythm, electrocardiogram, oxygen and carbon dioxide content of the blood, hemoglobin and hematocrit are made during the course of each

1003537205

experiment in dogs. The experiments with guinea pigs cannot be so well controlled because the size of the animal does not permit withdrawal of blood without interfering with the course of the experiment. The immobilization of the lung as reported by Irwing et al (Anatomical Record 119:391, 1954) and based on the Meltzer preparation (J. Exper. Med. 11:622, 1909) produces good oxygenation but significant carbon dioxide retention. Furthermore this method does not control the endotracheal pressure, our findings indicate increase in the pulmonary artery pressure and evidence of pulsatile flow when the oxygen endotracheal pressure is 12 cm. of water or more.

Our new method of immobilization of the lung permits controlled automatic respiration, because only one small area of the external surface of the lung is kept immovable. The illumination of the observed area is obtained with an incident light illuminator plus an additional reflection box such as commercially made for the metalurgic type of microscope. This method permits us to make observations anywhere on the surface of the lungs.

Our aim is to obtain basic line information under controlled conditions and then to compare these results with the ones obtained from animals submitted to chronic hypoxia.

Any vasomotor action on the pulmonary vessels can be directly observed after the injection of drugs such as adrenaline, acetylcholine, hexamethonium, serotonin and nicotine.

6. Budget Plan:	Salaries	4,800
	Expendable Supplies	1,400
	Permanent Equipment	2,400
	Overhead (15%)	*1,290
	Other	--
		*\$9,890

*-Recalculating the overhead with exclusion of the
\$2,400 for permanent equipment gives:
\$930.00 for overhead at 15%
\$9,530 Total

7. Anticipated Duration of Work: 3 years

8. Facilities and Staff Available:

The facilities of the Department of Pathology, University of Wisconsin Medical School. The staff available is formed by the director of the project, one full-time technical assistant and part-time student help. The equipment: 3 low pressure chambers; one x-ray machine with a fluoroscope for cardiac catheterization in dogs; one microscope with built-in direct illumination, one 16 mm. movie camera; one Grass multi-channel Recording equipment partially equipped; tables and rotary stage for animal surgery; two Van Slyke apparatus; one Sanborn electrocardiograph; one automatic respirator.

9. Additional Requirements:

Completion of the equipment for the recording apparatus. Parts for the Van Slyke apparatus. Cardiac catheters. One oxymeter. Equipment to determine pulmonary artery flow (under study).

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10. Additional Information:

A complete curriculum vitae of the director of the project is included. Histological studies of the total capillary bed in muscle, and myocardium and demonstration of succinicdehydrogenasa activity in different tissues from animals submitted to experimental hypoxia are currently investigated under financial support from the Wisconsin Heart Association.

Signature Enrique Valdivia, M.D.
Director of Project

A. W. Peterson
Vice President - Business and Finance

1003537207

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street New York 17, N. Y.

Application for Research Grant

#237A

Date: May 14, 1959

1. Name of Investigator: Dr. George B. Vetter, Professor of Psychology, N.Y.U.
Dr. Thomas N. Jenkins, Professor Emeritus of Psychology, N.Y.U.
2. Title: Dr. George B. Vetter, Project Administrator
Dr. Thomas N. Jenkins, Research Director
3. Institution & Address: New York University
New York 3, N. Y.
4. Project or Subject: Project No. 1: To determine the relationships between smoking habits and 131 primary personality qualities and basic personality syndromes. To find the differentiating personality traits of heavy, light and non-smokers, using the Jenkins global personality test, as a preliminary step in ascertaining whether biochemical individuality and its personality correlates are more or less important than smoking as predictors of morbidity rates or possibly even smoking habits.
5. Detailed Plan of Procedure:

A common fallacy is the assumption that correlation indicates a causal relationship. For this reason, specialists in research design repeatedly emphasize the fact that relationships are not necessarily causal. For instance, a correlation between smoking habits and heart disease does not show that smoking is the cause. It also would be erroneous to conclude that a predisposition for heart disease is the cause of heavy smoking. Again, suppose smoking habits are definitely related to personality patterns. It obviously would be erroneous to conclude that smoking is the cause of the personality pattern. In fact, we would be more likely to conclude that the personality pattern accounts to some extent for the heavy use of tobacco.

In recent years, evidence has been accumulating to support the view that basic personality traits are closely related to biochemical or humoral conditions in the body. There is also evidence that there is a causal relationship between basic personality variates and such biochemical conditions. Likewise, there is an important body of facts which suggest that biochemical conditions are causally related to cancer incidence.

Recently, Friedman and Rosenman (J. Am. Med. Assoc., March 21, 1959) showed that there may be significant relationships between overt behavior patterns and certain circulatory diseases (coronary disease and arcus senilis). But they also noted that the intense, goal-directed group of subjects, on the average, were the heavier cigarette smokers. Here again one might erroneously conclude that smoking is the cause of such circulatory diseases. But a careful analysis of the data for this sample indicated that tobacco consumption could not be the cause of coronary disease. "The data suggest that excessive smoking may be a characteristic of the overt behavior" of this sample, "but not a causal agent of the high incidence of cardiac disease found characteristic" of this behavior pattern.

There may be a significant, non-causal relationship between smoking habits and cancer incidence. But there may be an even more significant, and possibly causal, relationship between smoking habits and personality patterns. The preliminary purpose of our research program is to pinpoint the relationships which exist between smoking habits and primary personality traits. The global test of personality developed at New York University during the past 17 years furnishes measures of 131 primary personality qualities. Recently, scoring procedures were developed to obtain objective measures of the adrenergic and cholinergic syndromes which were factorially isolated by Jenkins (The neutral theory of personality, etc., Trans. N. Y. Acad. Sci., 1955, 17, 315-330).

PLAN OF RESEARCH PROCEDURE FOR PROJECT NO. 1

We will establish contact with as many as possible of 337 students already tested extensively and intensively with a variety of personality measures, including the Jenkins global test. The scoring of the tests taken by these students will be rechecked for accuracy. The smoking habits of these students will be recorded, both as of the present, and their recall in regard to their smoking habits at the time the tests were taken. These subjects will be classified on the basis of their smoking patterns, and the scores of the resultant groups compared on all test and personality trait scores.

6. Budget Plan:

Salaries	5,416.90
Expendable Supplies	2,235.00
Permanent Equipment	
Overhead (15%)	1,147.79
Other	
Total	\$8,799.69

7. Anticipated Duration of Work: Three months.

8. Facilities and Staff Available: Everything necessary to start immediately.

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of supply):

Signature George B. Vetter
 Thomas N. Jenkins
 Directors of Project

Sidney G. Roth
 Coordinator of Research Services

1003537209

RESEARCH PROJECT #1Survey and Retest of former Students.

(Budget of costs in addition to those in Program #1.)

Salaries:

Professor Thomas N. Jenkins (3 months)	3,000.00
Professor Geo. B. Vetter "	500.00
Statistical and clerical assistants (3 months)	1,736.00
OASI & TIAA payments	180.90
Total	\$5,416.90

Expendable Supplies:

Payments to test subjects (300 at \$5.00 each)	1,500.00
Personality test forms, rating scales, etc.	435.00
Rental: IBM Punch, Sorter, Verifier	300.00
Total	\$2,235.00

University Overhead (15%)	1,147.79
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GRAND TOTAL	\$8,799.69
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1003537210

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street New York 17, N. Y.

Application For Research Grant

#237B

Date: May 14, 1959

1. Name of Investigator: Dr. George B. Vetter, Professor of Psychology, N.Y.U.
Dr. Thomas N. Jenkins, Professor Emeritus of Psychology, N.Y.U.
2. Title: Dr. George B. Vetter, Project Administrator
Dr. Thomas N. Jenkins, Research Director
3. Institution New York University
& Address: New York 3, N. Y.
4. Project or Subject: Project No. 2: In addition to the basic objective outlined in PROJECT NO. 1, we propose to extend the investigation to determine the interrelationships between stability of personality traits, smoking habits, changes in smoking habits, and changes in environmental stresses.
5. Detailed Plan of Procedure:

The same 337 subjects already referred to in PROJECT NO. 1 would be retested and, at the same time, rated anew for smoking habits. Information also would be obtained regarding their personal history during the interim (one to two years) between initial testing and retesting. This operation would enable us to obtain the following results:

- (1) To determine retest reliability for 131 primary personality traits for various classes of smokers and for diverse smoking patterns.
- (2) To determine whether emotional crises or stresses during the interim between testing and retesting are related to changes in personality and smoking habits.
- (3) To determine whether relationships between environmental stresses and smoking would still exist after the influences of the personality variates are partialled out.

6. Budget Plan:

Salaries	3,412.50
Expendable Supplies	415.00
Permanent Equipment	
Overhead (15%)	574.13
Other	
Total	\$4,401.63

7. Anticipated Duration of Work: Three months.
8. Facilities and Staff Available: Working space, statistical machinery and clerical help available.

1003537211

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of supply):

Signature George B. Vetter
 Thomas N. Jenkins
 Director of Project

Sidney G. Roth
 Coordinator of Research Services

RESEARCH PROGRAM #2. (Three month project.)

Survey of Former Washington Square College Students.

SALARIES:

Statistical & Clerical assistance (3 months)	1,500.00
Professor Thomas N. Jenkins (3 months)	1,500.00
Professor Geo. B. Vetter (3 months)	300.00
	<u>3,300.00</u>
OASI payments	82.50
TIAA payments	30.00
Total	<u>3,412.50</u>

EXPENDABLE SUPPLIES:

Postage Stamps, stationery, rating blanks, etc.	115.00
Rental: IBM Punch, Sorter, Verifier (3 months)	300.00
Total	<u>3,827.50</u>
University Overhead (15%)	574.13

GRAND TOTAL \$4,401.63

1003537212

Anticipated Duration of Work: Three months.

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street New York 17, N. Y.

Application for Research Grant

#237A

Date: May 14, 1959

1. Name of Investigator: Dr. George B. Vetter, Professor of Psychology, N.Y.U.
Dr. Thomas N. Jenkins, Professor Emeritus of Psychology, N.Y.U.
2. Title: Dr. George B. Vetter, Project Administrator
Dr. Thomas N. Jenkins, Research Director
3. Institution: New York University
& Address: New York 3, N. Y.
4. Project or Subject: Project No. 1: To determine the relationships between smoking habits and 131 primary personality qualities and basic personality syndromes. To find the differentiating personality traits of heavy, light and non-smokers, using the Jenkins global personality test, as a preliminary step in ascertaining whether biochemical individuality and its personality correlates are more or less important than smoking as predictors of morbidity rates or possibly even smoking habits.

5. Detailed Plan of Procedure:

1003537213

A common fallacy is the assumption that correlation indicates a causal relationship. For this reason, specialists in research design repeatedly emphasize the fact that relationships are not necessarily causal. For instance, a correlation between smoking habits and heart disease does not show that smoking is the cause. It also would be erroneous to conclude that a predisposition for heart disease is the cause of heavy smoking. Again, suppose smoking habits are definitely related to personality patterns. It obviously would be erroneous to conclude that smoking is the cause of the personality pattern. In fact, we would be more likely to conclude that the personality pattern accounts to some extent for the heavy use of tobacco.

In recent years, evidence has been accumulating to support the view that basic personality traits are closely related to biochemical or humoral conditions in the body. There is also evidence that there is a causal relationship between basic personality variates and such biochemical conditions. Likewise, there is an important body of facts which suggest that biochemical conditions are causally related to cancer incidence.

Recently, Friedman and Rosenman (J. Am. Med. Assoc., March 21, 1959) showed that there may be significant relationships between overt behavior patterns and certain circulatory diseases (coronary disease and arcus senilis). But they also noted that the intense, goal-directed group of subjects, on the average, were the heavier cigarette smokers. Here again one might erroneously conclude that smoking is the cause of such circulatory diseases. But a careful analysis of the data for this sample indicated that tobacco consumption could not be the cause of coronary disease. "The data suggest that excessive smoking may be a characteristic of the overt behavior" of this sample, "but not a causal agent of the high incidence of cardiac disease found characteristic" of this behavior pattern.

There may be a significant, non-causal relationship between smoking habits and cancer incidence. But there may be an even more significant, and possibly causal, relationship between smoking habits and personality patterns. The preliminary purpose of our research program is to pinpoint the relationships which exist between smoking habits and primary personality traits. The global test of personality developed at New York University during the past 17 years furnishes measures of 131 primary personality qualities. Recently, scoring procedures were developed to obtain objective measures of the adrenergic and cholinergic syndromes which were factorially isolated by Jenkins (The neutral theory of personality, etc., Trans. N. Y. Acad. Sci., 1955, 17, 315-330).

PLAN OF RESEARCH PROCEDURE FOR PROJECT NO. 1

We will establish contact with as many as possible of 337 students already tested extensively and intensively with a variety of personality measures, including the Jenkins global test. The scoring of the tests taken by these students will be rechecked for accuracy. The smoking habits of these students will be recorded, both as of the present, and their recall in regard to their smoking habits at the time the tests were taken. These subjects will be classified on the basis of their smoking patterns, and the scores of the resultant groups compared on all test and personality trait scores.

6. Budget Plan:	Salaries	5,416.90
	Expendable Supplies	2,235.00
	Permanent Equipment	
	Overhead (15%)	1,147.79
	Other	
	Total	\$8,799.69

7. Anticipated Duration of Work: Three months.

8. Facilities and Staff Available: Everything necessary to start immediately.

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of supply):

Signature George B. Vetter
 Thomas N. Jenkins
 Directors of Project

Sidney G. Roth
 Coordinator of Research Services

1003537214

RESEARCH PROJECT #1

Survey and Retest of former Students.

(Budget of costs in addition to those in Program #1.)

Salaries:

Professor Thomas N. Jenkins (3 months)	3,000.00
Professor Geo. B. Vetter "	500.00
Statistical and clerical assistants (3 months)	1,736.00
OASI & TIAA payments	180.90
Total	<u>\$5,416.90</u>

Expendable Supplies:

Payments to test subjects (300 at \$5.00 each)	1,500.00
Personality test forms, rating scales, etc.	435.00
Rental: IBM Punch, Sorter, Verifier	300.00
Total	<u>\$2,235.00</u>

University Overhead (15%)

1,147.79

GRAND TOTAL \$8,799.69

1003537215

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street New York 17, N. Y.

Application For Research Grant

#237B

Date: May 14, 1959

1. Name of Investigator: Dr. George B. Vetter, Professor of Psychology, N.Y.U.
Dr. Thomas N. Jenkins, Professor Emeritus of Psychology, N.Y.U.
2. Title: Dr. George B. Vetter, Project Administrator
Dr. Thomas N. Jenkins, Research Director
3. Institution & Address: New York University
New York 3, N. Y.

4. Project or Subject: Project No. 2: In addition to the basic objective outlined in PROJECT NO. 1, we propose to extend the investigation to determine the interrelationships between stability of personality traits, smoking habits, changes in smoking habits, and changes in environmental stresses.

5. Detailed Plan of Procedure:

The same 337 subjects already referred to in PROJECT NO. 1 would be retested and, at the same time, rated anew for smoking habits. Information also would be obtained regarding their personal history during the interim (one to two years) between initial testing and retesting. This operation would enable us to obtain the following results:

- (1) To determine retest reliability for 131 primary personality traits for various classes of smokers and for diverse smoking patterns.
- (2) To determine whether emotional crises or stresses during the interim between testing and retesting are related to changes in personality and smoking habits.
- (3) To determine whether relationships between environmental stresses and smoking would still exist after the influences of the personality variates are partialled out.

6. Budget Plan:

Salaries	3,412.50
Expendable Supplies	415.00
Permanent Equipment	
Overhead (15%)	574.13
Other	
Total	\$4,401.63

7. Anticipated Duration of Work: Three months.

8. Facilities and Staff Available: Working space, statistical machinery and clerical help available.

1003537216

9. Additional Requirements: Second Street New York 100 11 11
10. Additional Information (Including relation of work to other projects and other sources of supply): University of Washington

Signature George B. Vetter
Thomas N. Jenkins
 Director of Project

Sidney G. Roth
 Coordinator of Research Services

Dr. Thomas N. Jenkins, Research Director

RESEARCH PROGRAM #2. (Three month project.)

Survey of Former Washington Square College Students.

SALARIES:

Statistical & Clerical assistance (3 months)	1,500.00
Professor Thomas N. Jenkins (3 months)	1,500.00
Professor Geo. B. Vetter (3 months)	300.00
	<u>3,300.00</u>
OASI payments	82.50
TIAA payments	30.00
Total	<u>3,412.50</u>

EXPENDABLE SUPPLIES:

Postage Stamps, stationery, rating blanks, etc.	115.00
Rental: IBM Punch, Sorter, Verifier (3 months)	300.00
Total	<u>3,827.50</u>
University Overhead (15%)	574.13

GRAND TOTAL \$4,401.63

1003537217

Application For Research Grant

56
*Looks good.
method of smoking not
covered.*
Date: January 7, 1955

1. Name of Investigator: **Joseph F. Volker, D.D.S., Ph.D.
Joseph P. Lazansky, M.D., D.M.D.**
2. Title: **Dean and Associate Dean**
3. Institution
& Address: **University of Alabama School of Dentistry
Medical Center
Birmingham, Alabama**
4. Project or Subject: **The Effects of Tobacco on Selected Oral Structures**

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

In 1953, the American public spent over one billion dollars for the prevention and treatment of oral disease. The expenditure of the majority of these funds resulted from the almost universal occurrence of dental caries and periodontal disease. In addition to these two major diseases, the mouth is the site of approximately 4% of all malignant growths and oral manifestations of systemic disease are frequent findings. Unfortunately, the factors associated with the initiation, modification and prevention of oral disease states are little understood. Although it is questionable that the use of tobacco may modify oral disease, such a possibility should be investigated, especially since the mouth has contact with tobacco in smoke, juice, and solid form in higher concentration than any other part of the human organism. Accordingly, we would like to investigate the possible effects of tobacco on selected oral structures.

Initially, the studies would be limited to the following areas:

1. The effect of tobacco on the oral flora

Long term studies on the ecology of the oral flora are being carried out at this institution by Frederick Kraus, M.D., D.M.D. It is proposed to modify his program to include observations on the influence of tobacco on oral ~~micro~~ microorganisms.

1003537218

2. The effect of tobacco on salivary secretions

Leon Schneyer, D.D.S., Ph.D., of this University is engaged in basic studies on salivary secretions. He has developed physical facilities that can be adapted to the study of the effects of tobacco on salivary gland secretion.

3. The effect of tobacco on the dental plaque of the enamel surface

W. Ward Pigman, Ph.D., of this faculty has made extensive studies of the biochemistry of the dental plaque and the enamel surface as they relate to the etiology of dental caries. Modification of his research program is contemplated to include a study of the aforementioned phenomenon.

4. The effect of tobacco smoking on the oral epithelium

Leonard Robinson, D.M.D., M.D., has made a continuing study of the normal and abnormal histology and biochemistry of tooth supporting tissues. These researches would be expanded to include observations on the effect of tobacco on the oral epithelium.

It should be noted that the proposed cooperative approach has many advantages. The investigators are in close physical proximity to one another and their educational backgrounds complement each other. Common use of controls, standardization of experimental procedures and a multiplicity of clinical observations on the same individuals under comparable clinical conditions anticipated.

1003537219

6. Budget Plan:

Two year estimate

Salaries	\$20,000
Expendable Supplies	2,000
Permanent Equipment	5,000
Overhead	3,000
Other travel, publication costs	1,000
Total	\$31,000

7. Anticipated Duration of Work: It is expected that two years would be needed to complete and evaluate the researches outlined above. Continuation beyond that period would depend on the nature of the findings and require the mutual agreement of the participants and the sponsor.

8. Facilities and Staff Available:

Dr. Frederick Kraus, Dr. Leonard Robinson, Dr. Ward Pigman and Dr. Leon Schneyer. Each of these persons has a laboratory adequately equipped for routine investigations. Technical assistants trained to carry out routine procedures are attached to each laboratory.

9. Additional Requirements:

It is estimated that the proposed investigation would require the equivalent of two full-time technical assistants and two part-time student assistants. Further expansion of the work would require supplementary funds.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The Medical College of Alabama and School of Dentistry of the University of Alabama sponsor joint basic science departments. The personnel enumerated above have the privilege of consultation with a considerable number of trained investigators in their own and other specialized fields. There is an adequate source of clinical material available for study. The Dental Clinic of the University Medical Center renders service to more than 30,000 patients per annum. The Medical Center Library makes available adequate bibliographic and reference sources. A preliminary survey of the pertinent literature has been completed.

Signature /s/ J. F. Volker, D.D.S. Ph.D.
Director of Project /s/ Joseph P. Lazansky, M.D.
D.M.D.

/s/ Harold A. Helms
Business Officer of the Institution Business Manager

1003537220

1003537221



Application For Research Grant

Date:

January 10, 1955

1. Name of Investigator: **Henry K. Wachtel, M.D.**
2. Title: **Scientific Director and President**
3. Institution & Address: **Chemical Hormone Corporation
670 Lexington Avenue
New York 22, New York**
4. Project or Subject: **Investigations concerning the relations existing between cancer disease and hormonal disturbances of the pituitary gland.**

The enclosed reprint from *Experientia* and the booklet, "The Role of the Pituitary in Cancer" review the studies already undertaken and include the references thereof.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

- A. The carcinogenic factor isolated from the pituitary gland, and reported in 1946 in *Science* (see the enclosed reprint), has been obtained in crystalline form. A single subcutaneous injection of 8 mg. of crystals dissolved in 0.2 cc. of sesame oil provokes cancer in the mouse within a period of about 3 months, following the injection. The crystals may be usually obtained in larger quantities. The average yield is about 0.015 mg from 1 pituitary gland of cattle.

The crystals will be tested concerning their carcinogenic properties on rabbits and dogs. A quantity of the crystals will be furnished to investigators indicated by the Committee for their studies.

Investigations will be undertaken to purify the crystals, with the view of establishing their chemical formula.

- B. The hormonal factor of the pituitary gland which in normal conditions prevents the carcinogenic action of the above-mentioned crystals and which in therapeutic tests reverses the trend of the disease was also obtained in crystalline form and was named Antineol. It is a steroid. Its usefulness in cancer therapy will be tested in a reputable hospital. If the Committee agrees to sponsor such tests, a joint application with the Hospital investigators will be filed.

1003537222

6. Budget Plan:

The subject "A"
will require \$24,000.
The subject "B" will
require \$18,000.

Salaries _____
Expendable Supplies _____
Permanent Equipment _____
Overhead _____
Other _____

Total _____

7. Anticipated Duration of Work:

Six months for Subject "A"; 12 months for
Subject "B".

8. Facilities and Staff Available:

For Subject "A", all facilities and staff of biochemical
and experimental laboratories, including animal research
facilities.

For Subject "B": all the facilities of a modern hospital.

9. Additional Requirements:

The above project will firmly establish the cause of cancer to be
connected with hormonal disturbances of the pituitary gland, and will
demonstrate the futility of theories which suspect the origin of cancer
to be from virus infection, tobacco smoking, and other hypothetical
agents.

10. Additional Information (Including relation of work to other projects and other sources of supply):

Signature _____

Director of Project

HENRY K. WACHTEL, M.D.

Business Officer of the Institution

1003537223

Application For Research Grant

Date: November 23, 1954

*out of my field
could be good
define "tobacco products"*

1. Name of Investigator: **Bernard M. Wagner, M.D.**
2. Title: **The Effect of Tobacco Derivatives on the Ground Substance**
3. Institution & Address: **Hahnemann Medical College and Hospital
235 North 15th Street
Philadelphia 2, Pennsylvania**
4. Project or Subject: **Relationship of tobacco products to vascular disease.**

1003537224

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Attention has again been centered on the adverse effects of smoking on the cardiovascular system. The exact nature of this phenomenon is not clear. It is well known that certain peripheral vascular diseases are made worse by smoking and may accelerate ~~xxxx~~ vascular occlusive events.

The earliest changes in blood vessels observed microscopically is a "thickening" of the intima. This covers a diverse range of events but in almost all vessels studied from patients with collagen disease, the first noticeable alteration is an accumulation of intimal ground substance. Following this change, a protein-rich material collects which is usually described as "hyaline" or "fibrinoid". Since the ground substance is intimately related to fibroblastic activity and circulating proteins, it becomes evident that these factors are worthy of study.

Investigations in this laboratory concerning the ground substance in the vessels of patients with generalized scleroderma, disseminated lupus erythematosus, malignant hypertension and acute rheumatic fever, have shown that the fibrinoid material present in each case is not identical. Thus, tinctorial similarity does not denote identity. In rheumatic fever, the collagen fibers are directly involved while in malignant hypertension the fibrinoid substance appears to be derived from the muscle.

Fibroblasts actively growing in tissue culture would serve as an ideal source of cells and ground substance. Pilot experiments (Mount Sinai Hospital, New York) have shown that mucopolysaccharides collect in the tissue culture liquor. Various tobacco products in solution would be applied to these cultures. These would then be studied for cytopathological changes and the culture media analyzed chemically for changes in protein-carbohydrate complexes. Cultures will be grown on thin pieces of sponge so that they can be fixed and sectioned. The sections will then be studied by histochemical methods to note any changes in the functional ability of the cells.

6. Budget Plan:

Salaries - Chemist, full time (Ph.D.)	\$5,500
Expendable Supplies	3,000
Permanent Equipment	3,000
Overhead	920 (8%)
Other	
Total	\$12,420

7. Anticipated Duration of Work: 12 to 18 months

8. Facilities and Staff Available:

Staff: Bernard M. Wagner, M.D., Assistant Professor of Pathology, in charge of Experimental Pathology

V. N. Damodaran, M.D., Instructor in Pathology

H. T. Segura, M.D., Research Fellow in Pathology

K. C. Pani, M.D., Research Assistant in Pathology

Sylvia Shapiro, Research Histopathology Technician

Facilities

Histochemistry Laboratory

Space for tissue culture laboratory

Animal Laboratory

Radioisotope Laboratory

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of supply):

At present, the Section of Experimental Pathology is being supported by grants from the Heart Association of Southeastern Penna., Office of the Surgeon General, U. S. Army and the Cardiovascular Institute, Hahnemann, Hospital. The problem under investigation concerns the nature of the ground substance in rheumatic fever. Histochemical, cytochemical, microchemical and biophysical methods are being used. The methods now established will allow for a comprehensive study of the effects of tobacco products on the pathogenesis of vascular disease. In addition to the vascular maladies previously mentioned, atherosclerosis will also be studied in the experimental animal. Tissue cultures from atherosclerotic rabbit aortas will be investigated as to the effects of tobacco products.

Signature _____

Director of Pathology Richard M. Wagner

Business Office Joseph J. Hayes, Jr.

1003537225

Application For Research Grant

Date: March 29, 1955

1. Name of Investigator: Sheppard M. Walker
2. Title: Associate Professor of Physiology
3. Institution & Address: University of Louisville School of Medicine
101 West Chestnut Street, Louisville 2, Kentucky

4. Project or Subject: Effect of smoking and of blood levels ^{of} tobacco products on the heart.
- I. Acute experiments. A comparison of reflex action and of direct action of tobacco derivatives on the induction of ventricular extrasystoles in the dog and in the human.
- II. Chronic experiments and long range observations. Effect on cardiac response in the dog and in the human of prolonged treatment with tobacco derivatives.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed): Acute experiments. For some time we have been looking for a reliable method for sensitization of the ventricles in the dog by stimulation of the cardiac sympathetic nerves. Now we have found that central sympathetic stimulation by that intracisternal injection of a mixture of mono- and dibasic potassium phosphate (0.03 cc./kg. of M/6 solution) does sensitize the ventricles to chloroform inhalation in vagotomized dogs previously anesthetized with sodium barbital. The chloroform under these conditions, produces ventricular extrasystoles. This method of sensitization by nerve stimulation has the advantage of being physiological, inasmuch as any trace of the potassium phosphate leaving the cerebrospinal fluid and entering the blood stream does so after the sympathetic stimulation is completed. Furthermore, we are able to induce the sensitization with sympathetic nerve stimulation alone, i.e., in vagotomized dogs. We plan to use this method of sensitization as a basis for comparative studies as follows: (1) Inhalation of tobacco smoke (previously collected in a Douglas bag) in one group of dogs. (2) Inhalation of filtered tobacco smoke in a second group of dogs. (3) Intravenous injection of tobacco derivatives, including nicotine and various irritants, in a third group of dogs. Similar comparative studies will be carried out in unsensitized dogs. In the human we plan to ~~also~~ observe the incidence of ventricular extrasystoles induced, by filtered and non-filtered smoking, in non-smokers and in inveterate smokers among the medical students before and after local anesthesia of the buccal cavity and the upper respiratory passages. Our rationale for these approaches to the problem of ventricular extrasystoles is based on the reports in the literature that smoking in man induces extrasystoles, while intravenous injection of nicotine in animals does not induce extrasystoles when the animal has previously been sensitized with agents like barium or chloroform.

1003537226

B. Chronic experiments and long range observations. We plan to house weanling puppies on the roof of the Medical School building and place a part of these puppies on diets containing various tobacco derivatives. During the course of development of these puppies we shall keep records of growth rates and food intakes for purposes of comparison with the litter mate controls. At regular intervals we shall obtain ECG recordings before and after stimulation with intracisternal potassium phosphate. The final experiment on these animals after they have reached maturity, will include observations on the effect of electrically induced ventricular tachycardia. Long range observations on the human will include ECG recordings, from non-smokers and smokers among medical students, taken annually and filed away for purposes of comparison over a period of years. The purpose of this study in man is to look for evidence of induction of cardiac damage within a single profession. Studies in the past on man have been primarily concerned with effects of smoking on the incidence of fatality in persons already showing cardiac damage, at the time the study was begun.

1003537227

6. Budget Plan:

Salaries	\$5,000.00
Expendable Supplies	2,000.00
Permanent Equipment	2,000.00
Overhead	820.00
Other - Travel	400.00
Total	\$10,220.00

7. Anticipated Duration of Work: Three years on studies with dogs. Many years on long range studies with man.

8. Facilities and Staff Available:

1. Facilities:
 - a. Grass EEG machine (4 channel)
 - b. Offner EEG machine (4 channel)
 - c. Kymograph for study of respiratory and blood pressure changes in the dog.
 - d. Douglas bags for inhalation studies in the dog.
 - e. Thyatron stimulator for electrical induction of tachycardia in the ventricles of the dog.
 - f. Sundry laboratory apparatus.

II. Staff: a. Sheppard M. Walker, b. animal caretaker to feed and weigh animals and

9. Additional Requirements: clean their living quarters, c. Drs. H. C. Lawson and J. P. Holt are available for consultation on interpretation of results.

- a. A research associate interested in our type of research and capable of helping me set up and carry out experiments. (I have in mind a man who has expressed interest in our research.)
- b. Dog cages and shelters on the roof of the Medical School building.
- c. Supplies of dogs and food.
- d. Supplies of EEG recording paper.
- e. Supplies of tobacco and tobacco derivatives.

10. Additional Information (Including relation of work to other projects and other sources of supply):

We are not aware of a current project that is related to the work we have proposed. We would like to state at this point what we believe to be the significance of our approach, as outlined in our proposed work. (A) We are studying, acutely, the combined effects of smoke inhalation and elevated sympathetic activity because we believe that irritative reflexes initiated in the mucous membrane of the buccal cavity and respiratory passages, together with central excitation associated with the act of smoking, are largely responsible for the extrasystoles observed in man. (B) In our long range studies designed to look for cardiac damage, the blood levels of tobacco derivatives in the puppies would be maintained at a considerably higher level than the levels of tobacco derivatives in the blood of inveterate smokers. If it should be shown that high blood levels of tobacco derivatives in dogs do not induce cardiac damage, then cardiac damage observed in inveterate smokers could not be attributed to blood levels of tobacco derivatives, without reservation.

Signature /s/ Sheppard M. Walker
Director of Project

Business Officer of the Institution

1003537228

Application For Research Grant

*CF. PHS #50
activated 2/1/55 renewed
2/1/56 & 2/1/57*
Date: June 28, 1957

1. Name of Investigator: **E. D. Warner, M. D., Professor of Pathology**
K. R. Cross, M. D., Lecturer, Pathology
2. Title: **Correlation of bronchial epithelial changes with comparable changes in other organs - A pathologic anatomic study.**
3. Institution
& Address: **Department of Pathology**
College of Medicine
The State University of Iowa
Iowa City, Iowa
4. Project or Subject: **We propose to study each consecutive autopsied case, attempting to correlate (1) the skin changes (senile elastosis, keratosis and malignant dysplasia) and (2) metaplastic changes in the bronchial mucosa, buccal mucosa, laryngeal mucosa, and mucosa from other organs (which is routinely available in our autopsy material) with the incidence of bronchogenic carcinoma.**

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

In addition to the 15 sections of bronchial mucosa which we have been customarily taking, we will obtain small skin biopsies from the face and hand, a biopsy of buccal mucosa, a section including both true and false vocal cords and a section from the urinary bladder near the ureteral orifices. Materials and histories from cases already collected may also be used.

We will continue to supplement our clinical histories by means of questionnaires mailed to the next of kin as well as through interviews with the patients since we have been gratified with the response to the former method and with the correlation between the two.

Our research team developed for Pathologic Anatomic Study of Human Bronchi is still intact so that we can proceed immediately with the project.

In view of the aging population which comprises the majority of our hospital census and with the high autopsy rate which we have been able to maintain, we feel that we have ideal source material for a project of this nature.

1003537229

6. Budget Plan:

Salaries	Technician	\$ 420
Expendable Supplies		500
Permanent Equipment		
Overhead	(8%)	80
Other		
Total		\$1000

7. Anticipated Duration of Work: One year

8. Facilities and Staff Available:

Our research team developed for Pathologic Anatomic Study of Human Bronchi is still intact so that we can proceed immediately with the project.

9. Additional Requirements:

This request is for \$1000 to supplement \$3000 of unexpended balance on hand. The total of \$4000 will be used to conduct the project outlined above.

10. Additional Information (Including relation of work to other projects and other sources of supply):

Signature _____
Director of Project **WARNER**

Business Officer **JON L. JONES** Business Manager and Secretary

1003537230

CROSS REFERENCE SHEET

Name or Subject

E. D. Wren

Regarding

50 FRI

SEE

K. R. Cross

1003537231

Application For Research Grant

OK E
smoking study.
Date: May 25, 1955

1. Name of Investigator:

Richard L. Wechsler, M. D.

2. Title:

Clinical Physiologist

3. Institution

& Address:

Montefiore Hospital Institute of Research
3459 Fifth Ave., Pittsburgh 13, Pennsylvania

4. Project or Subject:

Effect of Cigarette Smoking on Cerebral Blood Flow, Cerebral Metabolism, Blood Gases, Blood pH, Arterial Pulse Pressure Curves, Electrocardiograms, and Electroencephalograms.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Patients or paid subjects will be chosen at random from the hospital or student population. All will be people who smoke cigarettes, but a 12 hour period of abstinence from smoking will be observed. The studies will be accomplished in the morning with the subjects in a fasting state at bed rest in the supine position. A 30 minute rest period will precede the control or "before" studies. Thirty minutes will be allotted for smoking 3 cigarettes consecutively. After finishing the last one, the experimental or "after" studies will be carried out. The following studies will be accomplished before and after smoking, and each patient will act as his own control.

1. Cerebral Blood Flow using the N_2O Technique (Kety, S.S. The Quantitative Determination of Cerebral Blood Flow in Man, Methods in Medical Research, Year Book Publishers, Chicago, 1948, Vol. I, pp 204-215.
2. Arterial and Cerebral Venous O_2 and CO_2 contents by the manometric technique of Van Slyke and Neill, (Peters, J. A. and Van Slyke, D. D., Quantitative Clinical Chemistry, Williams and Wilkins, Baltimore, 1931).
3. Arterial and Cerebral Venous pH measured anaerobically at room temperature by means of a glass electrode and Cambridge potentiometer. Values will be corrected to $37^\circ C$ by the factors of Rosenthal (Effect of Temperature on pH of Blood and Plasma in Vitro, J. Biol. Chem., 1948, 173, 25).

(Continued on reverse side)

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- WECHSLER, R. L.: Development of a new method for continuous measurement of cerebral blood flow in humans under acceleration. Report No. NM 001 060.03.01 Phase I of study No. NM 001 060.03 titled "Effects of Acceleration upon Cerebral Metabolism and Cerebral Blood Flow," Aviation Medical Acceleration Laboratory, Naval Air Development Center, Johnsville, Pa. August 1952.
- Duane, T. D., WECHSLER, R. L., Ziegler, J. E., and Beckman, E. L.: Studies on cerebral Physiology of monkeys at 12 negative G. Report No. NM 001 060.03.03, Phase II of Study No. NM 001 060.03 titled "Effects of Acceleration upon Cerebral Metabolism and Cerebral Blood Flow," Aviation Medical Acceleration Laboratory, Naval Air Development Center, Johnsville, Pa. *J. Av. Med.* 23:479-489, Oct. 1952, (Presented at the Aero Medical Society Meeting, 1952).

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- WECHSLER, R. L., and Roth, J.: Measurement of the Rate of Gastric Emptying in Man as Determined by the Clearance of a Radioactive Colloid (AG I¹³¹): Normal Values, Effect of Urecholine Chloride and Morphine Sulfate, Federation Proceedings, 13:161, March, 1954. (Presented at the American Physiological Society Meetings, March 1954) and Am. J. Sc. (In press).
- WECHSLER, R. L., Crum, W. and Roth, J.: The Blood Flow and O₂ Consumption of the Human Brain in Hepatic Coma, Proceedings of the American Federation for Clinical Research, May 1954.
- Stone, H. H., MacKross, T. H., and WECHSLER, R. L., The Effects on Cerebral Circulation and Metabolism in Man of Acute Reduction in Blood Pressure by Means of Intravenous Hexamethonium Bromide and Head Up Tilt, Anaesthesiology, 16, 168, 1955.
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Continuation of Item #5.

4. Arterial and Cerebral Venous pCO_2 will be calculated by means of the nomograms of Peters and Van Slyke, (Peters, J. A. and Van Slyke, D. D., Quantitative Clinical Chemistry, Williams and Wilkins, Baltimore, 1931).

The following studies will be accomplished at short intervals every 2 to 4 minutes before, during, and after smoking.

5. Intraarterial Pulse Pressure Wave Recordings. A Sanborn Electro-manometer and Twin Viso Recorder will be used.
6. Electrocardiograms (Standard 12 leads with multiple recordings of Lead V_4). The Twin Viso Recorder will be used.
7. Electroencephalograms with a Grass Encephalograph.

Cerebral O_2 ~~consumption~~ consumption and cerebral vascular resistance will be calculated from this data.

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Continuation of Item #8.

(3) Staff

1. Richard L. Wechsler, M.D., Clinical Physiologist, 7 years experience in field of cerebral blood flow and metabolism. (Bibliography enclosed)
2. Yale David Koskoff, M.D., Ph.D., Director of Montefiore Hospital Institute of Research.
3. Chaskiel S. Grossman, M.D., Electroencephalographer. Will read electroencephalograms.
4. Richard Abrams, Ph.D., (Biochemistry). Associate Director, Montefiore Hospital Institute of Research.
5. Mr. Philip Louis Wolf, Research Assistant, trained in techniques necessary for accomplishing project.
6. Mr. Robert Hutchison, Research Assistant, trained in techniques necessary for accomplishing project.

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6. Budget Plan:

Salaries	\$ 5,400.00
Expendable Supplies	1,600.00
Permanent Equipment	2,000.00
Overhead	1,000.00
Other	
Total	\$10,000.00

7. Anticipated Duration of Work:

One Year.

8. Facilities and Staff Available:

(1) Source of Human Subjects

(2) Equipment

2 Van Slyke Manometric Gas Apparatus

1 Cambridge pH Meter

2 Grass Electroencephalographs

1 Hamilton Electromanometer (Sanborn)

1 Twin Viso Recorder (Sanborn)

Equipment for Cerebral Blood Flow Studies including gas mixtures, manifolds, syringes, and so on.

9. Additional Requirements:

None.

10. Additional Information (Including relation of work to other projects and other sources of supply):

Similar studies are in progress evaluating various anticholinergic compounds.

Signature /s./ Richard L. Wechsler
Director of Project

/s./ Yale David Koskoff, M.D.
Business Officer of the Institution

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#93R1

TOBACCO INDUSTRY RESEARCH COMMITTEE

350 Fifth Avenue, New York 1, N. Y.

Application for Research Grant

Date: April 27, 1956

1. Name of Investigator:

Richard L. Wechsler, M. D.

2. Title:

Clinical Physiologist

3. Institution & Address:

Montefiore Hospital Institute of Research
3459 Fifth Avenue, Pittsburgh 13, Pennsylvania

4. Project or Subject:

Effect of Cigarette Smoking and Intravenous Nicotine on Cerebral Blood Flow, Cerebral Metabolism, Blood Gases, Blood pH, Arterial Pulse Pressure Curves, Electrocardiograms, and Electroencephalograms in People of the Older Age Group with Arteriosclerosis. Studies during the past 9 months have failed to show any significant tobacco effects on cerebral blood flow or metabolism in young men. At the Tobacco Committee Conference in New York (March 16, 1956), the investigation of cigarette effects or intravenous nicotine effects on human brains of older people was discussed and suggested. In addition, the effects of denicotinized cigarettes will be studied.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Patients or paid subjects over 60 years of age will be chosen at random from the hospital or general population. All will be people who smoke cigarettes, but a 12 hour period of abstinence from smoking will be observed. The studies will be accomplished in the morning with the subjects in a fasting state at bed rest in the supine position. A 30 minute rest period will precede the control or "before" studies. Thirty minutes will be allotted for smoking 3 cigarettes consecutively. After finishing the last one, the experimental or "after" studies will be carried out. The following studies will be accomplished before and after smoking or before and during the injection of intravenous nicotine (nicotine bitartrate). Each patient will act as his own control.

1. Cerebral Blood Flow using the N₂O Technique (Kety, S. S. The Quantitative Determination of Cerebral Blood Flow in Man, Methods in Medical Research, Year Book Publishers, Chicago, 1948, Vol. I, pp 204-215.
2. Arterial and Cerebral Venous O₂ and CO₂ Contents by the manometric technique of Van Slyke and Neill, (Peters, J. A. and Van Slyke, D. D., Quantitative Clinical Chemistry, Williams and Wilkins, Baltimore, 1931).
3. Arterial and Cerebral Venous pH measured anaerobically at room temperature by means of a glass electrode and Cambridge potentiometer. Values will be corrected to 37° C by the factors of Rosenthal (Effect of Temperature on pH of Blood and Plasma in Vitro, J. Biol. Chem., 1948, 173, 25).
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6. Electrocardiograms (standard 12 leads with multiple recordings of Lead V_4). The Twin Viso Recorder will be used.
7. Electroencephalograms with a Grass Encephalograph. Cerebral O_2 consumption and cerebral vascular resistance will be calculated from this data.

6. Budget Plan:

Salaries	\$ 6,200.00
Expendable Supplies	1,600.00
Permanent Equipment	1,200.00
Overhead	1,000.00
Other	-----

TOTAL \$10,000.00

7. Anticipated Duration of Work:

One Year.

8. Facilities and Staff Available:

1. Source of Human Subjects

2. Equipment

- 2 Van Slyke Manometric Gas Apparatus
- 1 Cambridge pH Meter
- 2 Grass Electroencephalographs
- 1 Hamilton Electromanometer (Sanborn)
- 1 Twin Viso Recorder (Sanborn)

Equipment for Cerebral Blood Flow Studies including gas mixtures, manifolds, syringes, and so on.

3. Staff

- (1) Richard L. Wechsler, M. D., Clinical Physiologist, 8 years experience in field of cerebral blood flow and metabolism.
- (2) Yale David Koskoff, M. D., Ph. D., Director of Montefiore Hospital Institute of Research.
- (3) Philip Brostoff, M. D. Cardiologist.
- (4) Chaskiel Grossman, M. D., Electroencephalographer.
Will read electroencephalograms.
- (5) Richard Abrams, Ph. D. (Biochemistry) Associate Director,
Montefiore Hospital Institute of Research.

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- (6) Mr. Philip Louis Woolf, Research Assistant, trained in techniques necessary for accomplishing project.

9. Additional Requirements:

None.

10. Additional Information (Including relation of work to other projects and other sources of supply):

Similar studies are in progress evaluating various anticholinergic compounds.

Signature

Richard L. Wechsler M.D.

Director of Project

Y.D. Kosloff M.D.

Business Officer of the Institution

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TOBACCO INDUSTRY RESEARCH COMMITTEE

350 Fifth Avenue, New York 1, N. Y.

Application for Research Grant

Date: April 27, 1956

1. Name of Investigator:

Richard L. Wechsler, M. D.

2. Title:

Clinical Physiologist

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3459 Fifth Avenue, Pittsburgh 13, Pennsylvania

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Salaries	\$ 6,200.00
Expendable Supplies	1,600.00
Permanent Equipment	1,200.00
Overhead	1,000.00
Other	-----

TOTAL \$10,000.00

7. Anticipated Duration of Work:

One Year.

8. Facilities and Staff Available:

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2. Equipment

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- 1 Cambridge pH Meter
- 2 Grass Electroencephalographs
- 1 Hamilton Electromanometer (Sanborn)
- 1 Twin Viso Recorder (Sanborn)
- Equipment for Cerebral Blood Flow Studies including gas mixtures, manifolds, syringes, and so on.

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- (1) Richard L. Wechsler, M. D., Clinical Physiologist, 8 years experience in field of cerebral blood flow and metabolism.
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- (4) Chaskiel Grossman, M. D., Electroencephalographer.
Will read electroencephalograms.
- (5) Richard Abrams, Ph. D. (Biochemistry) Associate Director,
Montefiore Hospital Institute of Research.

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- (6) Mr. Philip Louis Woolf, Research Assistant, trained in techniques necessary for accomplishing project.

9. Additional Requirements:

None.

10. Additional Information (Including relation of work to other projects and other sources of supply):

Similar studies are in progress evaluating various anticholinergic compounds.

Signature

Richard L. Wachsler M.D.
Director of Project

Y. D. Kosloff M.D.
Business Officer of the Institution

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CONFIDENTIAL

TIRC Grant #93

Progress Report #1

Dr. Richard L. Wechsler
Montefiore Hospital Institute of Research

February 16, 1956

"The Effects of Cigarette Smoking on the Human Brain and Cardiovascular System"

We have completed five successful studies of the cerebral and cardiovascular effects of cigarette smoking and expect to accomplish further work. Therefore, we would appreciate any suggestions.

The cerebral effects of smoking tobacco have been described by many men. Some of these descriptions have stated that tobacco has a quieting and relaxing effect and some have stated it has a stimulating effect. However, few objective studies of these cerebral effects have been reported.

Methods

Paid subjects were chosen at random from the student population. They were young men varying in age from 17 to 23 years. Four were smokers (1 to 1 1/2 package per day) and one did not smoke. A 12 hour period of abstinence from smoking was required in all cases. Studies were accomplished in the morning, with the subjects in a fasting state in the supine position.

After the introduction of the needles and the application of the electrocardiographic and electroencephalographic leads, a 30 minute rest period was observed. Following this period, control observations were made. The subject was then instructed to smoke 3 consecutive cigarettes within 30 minutes. Four-fifths of each cigarette was consumed in 8 to 10 minutes. Only one brand of a normal length cigarette was used in an attempt to keep this factor constant. After finishing the last cigarette, experimental studies were accomplished. Electrocardiograms (lead II), electroencephalograms, and intra-arterial pulse pressure wave recordings were made at frequent (2 to 4 minute) intervals before, during and after smoking. Cerebral blood flows (1), arterial and cerebral blood gases (2) and pH (3) were measured before and from 1 to 10 minutes after finishing the third cigarette. Cerebral metabolism and cerebral vascular resistance were calculated as previously described (1). Arterial and cerebral venous pCO₂ were calculated by means of the nomogram of Peters and Van Slyke (2). Arterial O₂ capacity and saturation were determined (4).

Results and Discussion (See Table I)

Since we only have data on 5 cases at this time, a statistical analysis was not considered advisable. Certain trends are present. The variation in the changes of cerebral blood flow and cerebral metabolism

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indicates that there will be no significant change in these measurements. There is a consistent but small increase in pulse rate but not in blood pressure. Our continuous recordings of electrocardiograms and pulse pressure curves have not yet been analyzed. There were no other consistent changes noted in the parameters studied including cerebral arterio-venous oxygen difference, cerebral vascular resistance, cerebral respiratory quotient, mean arterial blood pressure, hemoglobin, arterial O_2 capacity, O_2 saturation, arterial and internal jugular O_2 , CO_2 , pH and pCO_2 .

In every case intermittent flattening appeared on the electroencephalographic records (Figure I). This flattening occurred only during smoking of cigarettes and lasted from 1 to 30 seconds. This flattening may be an abnormal attention response. Further analysis of the continuous electroencephalographic records by Dr. Chaskiel Grossman should clarify this problem.

Summary

In five normal young men the effects of smoking 3 normal sized cigarettes in 30 minutes were studied. Cerebral blood flows, cerebral metabolism, blood gases, blood pH, electrocardiograms, arterial pulse pressure curves, and electroencephalograms were accomplished before, during, and after smoking. Besides a consistent increase in the pulse rate and the consistent presence of intermittent flattening of the electroencephalographic recordings, no significant changes were noted.

References

1. Kety, S. S. and Schmidt, C. F. The Nitrous Oxide Method for the Quantitative Determination of Cerebral Blood Flow in Man; Theory, Procedure and Normal Values. J. Clin. Invest., 1948, 27, 476.
2. Peters, J. P. and Van Slyke, D. D. Quantitative Clinical Chemistry, Vol. II, Methods, Williams and Wilkins, Baltimore, 1932.
3. Rosenthal, T. B. Effects of Temperature on pH of Blood and Plasma Vitro. J. Biol. Chem., 1938, 126, 655.
4. Comroe, J. H., Jr. Methods in Medical Research, Vol. 2, p. 142, Year Book Publishers, Inc., Chicago, 1950.

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Table I a

Subject	Age	Remarks	Time 3rd cig to 2nd CBF	CBF cc/100 g/ min.		CMR O ₂ cc O ₂ /100 g/ min.		Vols. % (A-V) O ₂		CVR mm Hg/100 g/min.		CRQ	
				B	A	B	A	B	A	B	A	B	A
BY	20	Smokes about 1½ pack/day	10	94	54	5.9	3.0	6.3	5.6	0.9	1.6	1.06	1.00
CY	23	Smoke at least 1 pack/day	2	50	63	3.4	4.0	6.7	6.4	1.7	1.5	1.06	1.00
JB	19	Smoke at least 1 pack/day	6	52	61	3.7	4.4	7.2	7.2	1.5	1.4	0.97	0.97
JH	20	Used to smoke not for 2 yrs did not in- hale	8	72	40	3.9	3.2	5.5	8.1	1.2	2.2	1.00	1.00
MR	17	Smoke 1½ pack per day	1	72	72	3.8	3.9	5.3	5.4	1.2	1.2	1.00	1.02

Mean 68 58 4.1 3.7 6.2 6.5 1.3 1.6 1.02 1.00

Key - CBF--Cerebral Blood Flow
 CMR O₂--Cerebral Metabolic Rate (oxygen consumption)
 CVR--Cerebral Vascular Resistance
 CRQ--Cerebral Respiratory Quotient
 A--After Smoking
 B--Before Smoking

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Table I b

Sub- ject	O ₂ Content Vols. %				O ₂ Cap. Vols. %		O ₂ Sat. %		Pulse/min.		(mm. Hg.) MABP		Hb. gms/100 cc		CO ₂ Content Vols. %			
	Arterial B	A	B	A	B	A	B	A	B	A	B	A	B	A	Arterial B	A	B	A
BY	19.8	18.3	13.5	12.7	20.1	18.6	100	100	80	80	81	85	14.9	14.9	45.9	46.4	52.6	52.0
CY	19.2	19.7	12.5	13.3	20.5	21.4	95	93	60	75	83	95	15.4	15.9	46.5	47.8	53.6	54.2
JB	19.5	19.5	12.3	12.3	20.5	20.6	97	96	72	80	80	85	15.1	15.1	45.5	45.5	52.5	52.5
JH	19.5	19.7	14.0	11.6	21.5	20.9	92	95	84	96	88	87	15.3	15.3	47.6	44.9	53.1	53.0
MR	17.9	18.2	12.6	12.6	19.0	19.7	96	94	74	90	91	88	14.5	14.9	49.2	49.2	54.5	54.7
Mean	19.2	19.1	13.0	12.5	20.3	20.2	95	96	74	84	85	88	15.0	15.2	46.9	46.8	53.3	53.3

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Table I c

Sub- ject	pH				pCO ₂ mm Hg				E E G
	Arterial B	A	Venous B	A	B	A	B	A	
BY	-	-	-	-	-	-	-	-	Intermittent flattening lasts 4 to 30 seconds, flattening duration decreased as smoking continued. Started 3 minutes after first cigarette started. Flattening stopped within 3 to 12 seconds after cigarette.
CY	7.40	7.41	7.36	7.37	40	40	50	50	Intermittent flattening lasts 3 to 15 seconds. Started 25 seconds after first cigarette started. Flattening continued for 10 minutes after last cigarette.
JB	7.42	7.42	7.38	7.38	38	38	48	48	Intermittent flattening lasts 5 to 10 seconds. Started 1 min. after starting first cigarette. Flattening stopped within 30 seconds of last cigarette.
JH	7.38	7.41	7.31	7.35	43	38	56	51	Intermittent flattening lasts 1 to 3 seconds, starting 4 minutes after starting first cigarette. No inhaling.
MR	7.42	7.40	7.37	7.37	40	42	50	50	Intermittent flattening lasts 5 seconds. Started over a minute after start of first cigarette. 8/second alpha control and 9/second during smoking.
Mean	7.40	7.41	7.35	7.37	40	39	51	50	

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IIRC Grant #93

Progress Report #2
May, 1956

CONFIDENTIAL

THE EFFECTS OF CIGARETTE SMOKING ON THE HUMAN BRAIN AND CARDIOVASCULAR SYSTEM

By

Richard L. Wechsler, M. D.

Philip L. Woolf

Philip Brostoff, M. D.

Montefiore Hospital Institute of Research

Pittsburgh, Pennsylvania

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The cerebral effects of smoking tobacco have been described by many men. Some of these descriptions have stated that tobacco has a quieting and relaxing effect and some have stated it has a stimulating effect. However, few objective studies of these cerebral effects have been reported.

Methods

Paid subjects were chosen at random from the student population. They were young men varying in age from 17 to 24 years. Five were smokers (1 to 1½ package per day) and 2 did not smoke. A 12 hour period of abstinence from smoking was required in all cases.

Studies were accomplished in the morning, with the subjects in a fasting state in the supine position.

After the introduction of the needles and the application of the electrocardiographic and electroencephalographic leads, a 30 minute rest period was observed. Following this period, control observations were made. The subject was then instructed to smoke 3 consecutive cigarettes within 30 minutes. Four-fifths of each cigarette was consumed in 8 to 10 minutes. Only one brand of a normal length cigarette was used in an attempt to keep this factor constant. After finishing the last cigarette, experimental studies were accomplished. Electrocardiograms (lead II), electroencephalograms, and intraarterial pulse pressure wave recordings were made at frequent (2 to 4 minute) intervals before, during and after smoking. Cerebral blood flows (1), arterial and cerebral blood gases (2) and pH (3) were measured before and from 1 to 10 minutes after finishing the third cigarette. Cerebral metabolism and cerebral vascular resistance were calculated as previously described (1).

Arterial and cerebral venous pCO₂ were calculated by means of the nomogram of Peters and Van Slyke (2). Arterial O₂ capacity and saturation were determined (4).

Results and Discussion (See Table I)

The only changes noted were a statistically significant increase in pulse rate and a consistent change in the electroencephalographic patterns. In all 7 cases, an intermittent flattening appeared on the electroencephalographic recordings (see Figure I). This

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flattening occurred only during smoking of cigarettes and lasted from 1 to 30 seconds. Even in the 2 individuals who did not inhale, this flattening occurred but was shorter in duration. Dr. Chaskiel Grossman, our electroencephalographer, thought it was impossible to determine if the flattening was caused by the cigarette or was merely an abnormal attention response.

In one of the young men, electrocardiograms revealed a biphasic T wave 1 minute after the start of the first cigarette. Four minutes later the T waves became flat. Three minutes after the third cigarette the T waves returned to normal. In another individual a sinus arrhythmia occurred. These changes have been reported by others.

There were no significant changes in cerebral blood flow, cerebral metabolism, cerebral arteriovenous oxygen difference, cerebral vascular resistance, cerebral R. Q., hemoglobin, arterial O_2 capacity, O_2 saturation, arterial and internal jugular O_2 , CO_2 , pH and pCO_2 . The lack of significant change in mean arterial blood pressure has also been previously reported.

It is possible to attribute the lack of significant changes in cerebral hemodynamics and metabolism in this youthful group to the good condition of their cardiovascular systems. Therefore, it is thought to be of interest and importance to repeat these studies in individuals of the older age group (over 60 years). In addition, intravenous nicotine would allow the accomplishment of these studies during, instead of after, administration of the drug. The use of denicotinized cigarettes may help to determine the cause of the intermittent flattening found on the electroencephalographic records.

Summary

In 7 normal young men the effects of smoking 3 normal sized cigarettes in 30 minutes were studied. Cerebral blood flows, cerebral metabolism, blood gases, blood pH, electrocardiograms, arterial pulse pressure curves, and electroencephalograms were accomplished before, during, and after smoking. Besides a significant increase in the pulse rate and the consistent presence of intermittent flattening of the electroencephalographic recordings, no significant changes were noted. The value of repeating these studies in older people and with intravenous nicotine or denicotinized cigarettes is discussed.

1003537251

References

1. Kety, S. S. and Schmidt, C. F. The Nitrous Oxide Method for the Quantitative Determination of Cerebral Blood Flow in Man; Theory, Procedure and Normal Values. J. Clin. Invest., 1948, 27, 476.
2. Peters, J. P. and Van Slyke, D. D. Quantitative Clinical Chemistry, Vol. II, Methods, Williams and Wilkins, Baltimore, 1932.
3. Rosenthal, T. B. Effect of Temperature on pH of Blood and Plasma Vitro. J. Biol. Chem., 1938, 126, 655.
4. Comroe, J. H. Jr., Methods in Medical Research, Vol. 2, p. 142, Year Book Publishers, Inc., Chicago, 1950.

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Table I a

Sub- ject	Age	Remarks	Time 3rd Cig to 2nd CBF	CBF cc/100 g/min.		CMR O ₂ cc O ₂ 100 g/min.		(A-V) O ₂ Vols %		CVR mm Hg/100 g/min.		CRQ	
				B	A	B	A	B	A	B	A	B	A
WY	20	Smokes ab- out 1½ pack per day	10	94	54	5.9	3.0	6.3	5.6	0.9	1.6	1.06	1.00
CY	23	Smokes at least 1 pack/day	2	50	63	3.4	4.0	6.7	6.4	1.7	1.5	1.06	1.00
JB	19	Smokes at least 1 pack/day	6	52	61	3.7	4.4	7.2	7.2	1.5	1.4	0.97	0.97
JH	20	Used to smoke. Not for 2 yrs Did not inhale	8	72	40	3.9	3.2	5.5	8.1	1.2	2.2	1.00	1.00
MR	17	Smokes 1½ pack/day	1	72	72	3.8	3.9	5.3	5.4	1.2	1.2	1.00	1.02
MM	23	Non-smoker Did not inhale	3	47	45	3.1	3.3	6.5	7.4	1.8	1.8	1.01	1.07
RS	24	Smokes 1 pack/day	4	55	47	3.9	4.0	7.0	8.6	1.5	1.7	1.01	.89
Mean				63	55	4.0	3.7	6.4	7.0	1.4	1.6	1.02	.99
SE				6.4	4.3	0.3	0.2	0.3	0.4	0.1	0.1	0.01	0.02

Key - CBF--Cerebral Blood Flow

CMR O₂--Cerebral Metabolic Rate (oxygen consumption

A--After Smoking

B--Before Smoking

CVR--Cerebral Vascular Resistance

CRQ--Cerebral Respiratory Quotient

SE--Standard Error

1003537253

Table I b

Subject	O ₂ Content Vols. %				O ₂ Cap. Vols. %		O ₂ Sat. %		Pulse/min.		MABP mm. Hg.		Hb. gms/100 cc		CO ₂ Content Vols. %			
	Arterial		Venous		B	A	B	A	B	A	B	A	B	A	Arterial		Venous	
	B	A	B	A											B	A	B	A
BY	19.8	18.3	13.5	12.7	20.1	18.6	100	100	80	80	81	85	14.9	14.9	45.9	46.4	52.6	52.0
CY	19.2	19.7	12.5	13.3	20.5	21.4	95	93	60	75	83	95	15.4	15.9	46.5	47.8	53.6	54.2
JB	19.5	19.5	12.3	12.3	20.5	20.6	97	96	72	80	80	85	15.1	15.1	45.5	45.5	52.5	52.5
JH	19.5	19.7	14.0	11.6	21.5	20.9	92	95	84	96	88	87	15.3	15.3	47.6	44.9	53.1	53.0
MR	17.9	18.2	12.6	12.6	19.0	19.7	96	94	74	90	91	88	14.5	14.9	49.2	49.2	54.5	54.7
MM	19.0	19.4	12.4	12.0	20.4	20.7	95	95	68	77	84	82	16.0	16.0	47.5	45.7	54.0	53.6
RS	19.0	18.6	12.0	10.0	20.0	19.7	96	96	60	76	81	81	14.5	14.4	45.6	43.2	52.7	50.8
Mean	19.1	18.9	12.8	12.1	20.3	20.2	96	96	71	82	84	86	15.1	15.2	46.8	46.1	53.3	53.0
SE	0.2	0.3	0.2	0.4	0.3	0.3	1	1	3.4	3.0	1.5	1.7	0.2	0.2	0.5	0.7	0.3	0.5

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Table 1 c

Sub- ject	pH				C V	pCO ₂ mm Hg		A	E E G
	Arterial		Venous			A	B		
	B	A	B	A	B	A	B	A	
BY	-	-	-	-	-	-	-	-	Intermittent flattening lasts 4 to 30 seconds, flattening duration decreased as smoking continued. Started 3 minutes after first cigarette started. Flattening stopped within 3 to 12 seconds after cigarette.
CY	7.40	7.41	7.36	7.37	40	40	50	50	Intermittent flattening lasts 3 to 15 seconds. Started 25 seconds after first cigarette started. Flattening continued for 10 minutes after last cigarette.
JB	7.42	7.42	7.38	7.38	38	38	48	48	Intermittent flattening lasts 5 to 10 seconds. Started 1 min. after starting first cigarette. Flattening stopped within 30 seconds of last cigarette.
JH	7.38	7.41	7.31	7.35	43	38	56	51	Intermittent flattening lasts 1 to 3 seconds, starting 4 minutes after starting first cigarette. No inhaling.
MR	7.42	7.40	7.37	7.37	40	42	50	50	Intermittent flattening lasts 5 seconds. Started over a minute after start of first cigarette. 8/second alpha control and 9/second during smoking.
MM	7.41	7.41	7.35	7.37	40	38	52	49	Two seconds of flattening after start of first cigarette. Seven second flattening at end of first cigarette. During second cigarette 4 second intermittent flattening. None without cigarette. Intermittent flattening during third cigarette. Decreased amplitude of alpha waves.
RS	7.42	7.43	7.41	7.39	38	35	44	42	Intermittent flattening lasts 2 to 6 seconds.

Mean 7.41 7.41 7.36 7.37 40 39 50 48

SE 0.01 0.01 0.01 0.01 0.7 0.9 1.5 1.2

1003537255

W.Y. AGE 20

2

1 sec

100

LPF

RPF

LC

RC

LO

RO

LAT

RAT

1003537256

CONTROL DURING SMOKING
EFFECT OF CIGARETTE SMOKING ON ELECTROENCEPHALOGRAPHIC PATTERN

#218

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

Application For Research Grant

Date: November 11, 1958

1. Name of Investigator: **Russell W. Weller, M.D.**
2. Title: **Pathologist**
3. Institution: **Ephrata Community Hospital, Ephrata, Penna.**
& Address: **Chester County Hospital, West Chester, Penna.**
4. Project or Subject: **A Selected, Extended and Detailed Study of Human Bronchial Mucosa.**

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):
Selected lungs as listed below would be re-studied in a much more detailed manner by dissecting continuous, longitudinal sections (2.0 x 0.4 cm) as shown on the enclosed diagram. Utilizing this extensive bronchial mucosal area (approximately 200 sections each case) we could accurately map the extent and degree of cytological change in the following specially selected groups of persons chosen from our present series.

A.	Rural, male, non-smokers, over 40,	Normal mucosa (5 cases).
B.	" " " " " "	Metaplastic " "
C.	" " Moderate " " "	Normal " "
D.	" " " " " "	Metaplastic " "
E.	" " Heavy " " "	Normal " "
F.	" " " " " "	Metaplastic " "

G, H, I, J, K, L - Similar groups to above but living in heavily populated, urban areas last 10 years of life.

M to Z-12 groups similar to A to L but consisting of females.

Portions of these lungs could also be sent for chemical study to Doctor Artt (inorganic) and Doctor Kotin (organic).

1003537257

The 120 cases could probably be completed in 18 to 24 months.

This study would compare favorably in scope to that of Auerbach et al. It would give us a less-shaded picture of the incidence of "Carcinoma in Situ" which as presented by Auerbach, along with his large percentage of lung cancer cases, appears to many competent, unprejudiced observers to be an exceedingly "liberal" interpretation of early cancer and pre-cancerous growth changes.

1003537258

6. Budget Plan:

24 months

Salaries

(24 months)

\$15,000.00

Expendable Supplies

2,500.00

Permanent Equipment

1,550.00

Overhead 15%

Overhead

Other

Total

\$20,150.00

7. Anticipated Duration of Work:

24 months.

8. Facilities and Staff Available:

- A. Three rural hospitals with total of 800 beds (Sparta, Chester County, Lancaster General)
- B. One urban hospital of 600 beds (Hahnemann-Paoli.)
- C. Two tissue technicians, typist-secretary, medical student and record room librarian history-coordinators.
- D. Marge Deemer.

9. Additional Requirements:

None

10. Additional Information (Including relation of work to other projects and other sources of supply):

Signature /s./ Russell W. Miller

Director of Project

/s./ Nancy W. Appinall, Administrator

Business Officer of the Institution

1003537259

Committee:
Reimann, Chm.
Lynch
Kotin

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street

New York 17, N.Y.

#218R1

Activated 4/15/59

Application For Research Grant Renewal

Date: February 1, 1960

1. Name of Investigator: Russell W. Weller, M.D.
2. Title: Pathologist
3. Institution & Address: Memorial Hospital of Chester County
Chester County Hospital
(both in West Chester, Pennsylvania)
4. Project or Subject: A Complete and Detailed Microscopic and Medical
Historical Study of Human Bronchial Mucosa from
Autopsied Patients.
5. Detailed Plan of Procedure:

Selected series of lungs from rural and urban adult smokers and non-smokers are studied microscopically by dissection and blocking of cross sections of the distal trachea and the complete bronchial tree as illustrated on the enclosed diagram (200 to 250 sections from each case). *(see Progress Report attachment)*

The changes and their anatomical locations are recorded.

This study, although in some ways comparable to that of Auerbach et al, would appear to be more complete in evaluation of pathological changes and will probably provide material for re-evaluation of the atypical hyperplastic and early malignant changes so frequently observed by the above investigators.

6. Budget Plan:

Salaries	\$ 8,000.00
Expendable Supplies	1,250.00
Permanent Equipment	-----
Overhead (10%)	925.00
Other	-----
Total	\$10,175.00

7. Anticipated Duration of Work: 12 months
8. Facilities and Staff Available: As in original application (12.21.56)

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9. Additional Requirements: -----
10. Additional Information (Including relation of work to other projects and other sources of supply): -----

/s/ Russell W. Weller
Director of Project

/s/ Fred W. Schmid, Administrator
Business Officer of the Institution

1003537261

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

RENEWAL
Application For Research Grant

PAS #50 A R2
Initiated Feb. 1, 1955
Renewed Feb. 1, 1956

Date: 12-21-56

1. Name of Investigator: **Russell W. Weller, M.D.**

2. Title: **Pathologist**

3. Institution
& Address: **Ephrata Community Hospital, Ephrata, Pa.**
Chester County Hospital, West Chester, Pa. (Assistant)
Hahnemann Medical College, Philadelphia (Associate Prof.)

4. Project or Subject: **Pathologic Anatomic Study of Cellular Changes in Human Lungs.**

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

See previous outline.

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6. Budget Plan:

Salaries	<u>\$2900.00</u>
Expendable Supplies	<u>225.00</u>
Permanent Equipment	<u>93.00</u>
Overhead (25%)	<u>830.00</u>
Other	<u>100.00</u>
Total	<u>\$ 4148.00</u>

7. Anticipated Duration of Work:

Six months.

8. Facilities and Staff Available:

- A. Three rural hospitals with total of 800 beds
(Ephrata, Chester County, Lancaster General)
- B. One urban hospital of 600 beds (Hahnemann - Phila.)
- C. Two tissue technicians, typist-secretary, medical
student and record room librarian history - coordinators.
- D. Morgue diener.

9. Additional Requirements:

None

10. Additional Information (Including relation of work to other projects and other sources of supply):

See enclosed outline.

Signature /s/ Russell W. Weller
Director of Project

/s/ Nancy W. Aspinall, Administrator
Business Officer of the Institution

1003537263

No comment

(Address to whom the Application For Research Grant should be sent)

TOBACCO INDUSTRY RESEARCH COMMITTEE

(Name of person to whom the grant is to be made)

(Title of the study of which the grant is to be made) **July 29, 1954**

(Name of the person to whom the grant is to be made)

(Address of the person to whom the grant is to be made)

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In view of the recent statements issued by the American Cancer Society regarding statistical correlations between smoking and various pulmonary and cardiovascular diseases the applicant believes a more detailed morphological study as herein outlined is indicated.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

I. Classification of Persons Studied

- A. Those residing in heavily industrialized (smoke-polluted) areas at least two years.
- B. Those residing in heavily populated but non-industrialized areas at least two years who have never lived in area A.
- C. Those residing in moderately populated, non-industrialized areas at least two years who have never lived in areas A or B.
- D. Those residing in slightly populated rural areas for entire lifetime.
- E. Heavy smokers (more than 20 cigarettes, 4 cigars or 6 pipefuls daily).
- F. Moderate smokers (more than 10 cigarettes, 2 cigars or 3 pipefuls daily).
- G. Light smokers (less than 10 cigarettes, 2 cigars or 3 pipefuls daily).
- H. Non-smokers (who have never smoked).
- I. Occupation (listed as farmer, machinist, etc. & according to inhalants as given below).
 1. Organic dusts
 2. Inorganic dusts
 3. Organic vapors (each dust or vapor also listed)
 4. Inorganic vapors specifically
 5. Combinations of above
 6. None of above

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J. Period of time exposed (dates and number of months)

K. Sex and color

L. Age (limited to persons 14 years and older)

II. Pathological Study of Bronchi and Lungs (grossly and microscopically)
July 29, 1954

Twenty-one sections from bronchi and adjacent lung tissue are dissected and studied in each case employing the method outlined by applicant in "American Journal of Clinical Pathology", Vol. 23, No. 8, August 1953 with special emphasis on bronchial epithelial growth changes as hyperplasia, metaplasia and neoplasia.

III. Pathological Study of Heart (grossly and microscopically)

Weight of heart

Thickness of left and right ventricular walls

Three cross sections of left coronary artery (0.5cm from ostium, 3cm from ostium in circumflex branch, 7cm from ostium in posterior descending branch)

Three cross sections of right coronary artery (0.5cm from ostium, 3cm from ostium in circumflex branch, 7cm from ostium in posterior descending branch)

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IV. Source and Volume of Study Material
Material obtained from the University of Pennsylvania Hospital and the University of Pennsylvania School of Medicine, Philadelphia, within 20 miles of Philadelphia.

Approximately 350 autopsies per year.

In view of the recent statements issued by the American Cancer Society regarding statistical correlations between smoking and various pulmonary and cardiovascular diseases the applicant believes a more detailed morphological study as herein outlined is indicated.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed)

I. Classification of Persons Studied

A. Those residing in heavily industrialized (smoke-polluted) areas at least two years.

B. Those residing in heavily industrialized but non-polluted areas at least two years who have never lived in area A.

C. Those residing in moderately industrialized, non-polluted areas at least two years who have never lived in area A or B.

D. Those residing in slightly industrialized rural areas for entire lifetime.

E. Heavy smokers (more than 20 cigarettes, 4 cigars or 6 pipefuls daily).

F. Moderate smokers (more than 10 cigarettes, 2 cigars or 3 pipefuls daily).

G. Light smokers (less than 10 cigarettes, 2 cigars or 3 pipefuls daily).

H. Non-smokers (who have never smoked).

I. Occupations (listed as follows: machinist, etc. according to industrial classification).

1. Organic dusts

2. Inorganic dusts

3. Organic vapors (each dust or vapor also listed specifically)

4. Inorganic vapors

5. Combinations of above

6. None of above

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6. Budget Plan:

Salaries	\$5400.00
Expendable Supplies	\$1375.00
Social Worker personnel expenses	\$ 800.00
Travel Allowance	\$ 606.00
Overhead	
Other	
Total	\$8181.00

7. Anticipated Duration of Work: 12 months as pilot study

8. Facilities and Staff Available: Medical school and 600 bed hospital.
One full-time social worker
One half-time technician

9. Additional Requirements:

10. Additional Information (including relation of work to other projects and other sources of supply):

Signature Russell W. Weller /s/
Director of Project

Business Officer of the Institution

1003537266

CONFIDENTIAL

TIRC Grant #218 (Cf. #50A - PAS Study)

Report No. 1

Russell W. Weller, M.D.
Memorial Hospital of Chester County

February, 1960

A Selected, Extended and Detailed Study of Human Bronchial Mucosa

December 2, 1959

In our present study, we are dissecting the entire tracheo-bronchial tree and blocking all sections for histologic study. Depending on the size of the patient, the number of slides prepared varies, therefore, from approximately 200 to 250 per case. The material studied represents tracheobronchial epithelium from patients living in rural areas, suburban areas and medium sized cities at the present time. Our overall incidence of metaplasia to date is approximately 40% and of hyperplasia approximately 20%. In our non-smokers, our present incidence of metaplasia is approximately 30% and of hyperplasia 40%. In smokers (more than one package of cigarettes a day), the incidence of metaplasia is approximately 50% and of hyperplasia less than 10%. In none of our cases to date, in the present study which was begun in May, 1959, have we found carcinoma in situ, in spite of the fact that the entire tracheobronchial tree was dissected, and also that several of the cases of metaplasia were markedly atypical and would appear to present cellular abnormalities which at least approach some of those described by Auerbach as carcinoma in situ.

To date, we have prepared approximately 1200 slides and again, in order to eliminate cases immediately in which the bronchial epithelium is not suitable for study, we have not included in the above series any lungs in which there was any appreciable degree of autolysis or mechanical artefacts noted in the tracheobronchial epithelium. At this time, we feel rather strongly that the present study, because of the larger number of sections taken, the elimination of cases where any appreciable artefact is present, the inclusion of equal numbers of smokers and non-smokers and the more rigid and more acceptable interpretation of what constitutes carcinoma in situ, will be more generally scientifically acceptable, than the studies that have been performed in past, such as the much publicized Auerbach Report.

February 1, 1960

After approximately two months of anatomical study and experimentation in complete dissection of the tracheobronchial tree the method of coding and localizing of bronchial sections was, we feel, improved considerably (see enclosed diagram) over that used by Auerbach. We also worked out a screening process by which cases displaying autolysis or other physical, chemical or pathological changes rendering the bronchial epithelium unfit for accurate microscopic study were eliminated early in the dissection of a lung, and prior to the time a great deal of useless work had been performed. The method of reporting was also changed to individual descriptions of each bronchial section and surrounding lung, vascular and lymphoid tissues (see attached sample sheets). This latter method makes it possible to code and pinpoint, without time-consuming review, all pathological changes in all sections of each lung.

circulate at meeting

1003537267

Russell W. Weller, M.D.

Report No. 1

To date 28 cases are under study (approximately 6000 slides) of which approximately 12 cases (2400 slides) are completed. 13 cases (2800 slides) are partially completed. In ten additional cases (approximately 700 slides) we have decided the bronchial mucosa is unfit for accurate study and these cases and slides are therefore eliminated. Although this appears to be a slow and tedious process I feel after several years it will yield results which can be reviewed without difficulty by any qualified pathologist. Our findings should also help provide answers to many of the serious questions raised about the Auerbach study by recognized pathologists.

We do not feel a sufficient number of cases have been completed to date to justify any statistical evaluation although our results to date are essentially similar to those outlined during our August meeting in New York and in my letter of December 2, 1959 (preceding).

1003537268

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

\$183A
(CR. \$153)

Application For Research Grant

Date: November 24, 1958

1. Name of Investigator:

ROE E. WELLS, JR., M.D.

2. Title:

Associate in Medicine, Peter Bent Brigham Hospital; Clinical Associate in Medicine, Harvard Medical School

3. Institution

& Address:

Peter Bent Brigham Hospital, 721 Huntington Avenue, Boston, Massachusetts

4. Project or Subject:

Studies of the Antinicotinic action of the benzyl analog of serotonin and similar antiserotonin drugs.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Studies will be carried out on patients with chronic pulmonary disease, patients with arteriosclerotic heart disease and normal subjects. Recordings of pulse, blood pressure, minute ventilation, pulmonary compliance and flow resistance (intra-esophageal balloon technique) and in certain cases electroencephalograms will be carried out as follows: Normal subjects and patients will be studied at rest; after initial baseline observations either intravenous nicotine (1 to 3 mg. intravenously) will be administered or patient given two cigarettes to smoke in measured period of time followed by repeat studies within 2 minutes and of no nicotine or cigarette. The benzyl analog of serotonin (1-Benzyl-2-methyl-5-methoxy tryptamine hydrochloride) (BAS) will be given intramuscularly or by mouth (0.25 to 0.5 mg./kg) and studies repeated at 30 minute and 60 minute intervals. The entire study will also be carried out again using BAS prior to nicotine or cigarette smoking to ascertain any blocking effect. (see p10 Additional information)

Chronic heavy smokers (60 cigarettes a day and over) as well as non smokers will also be studied during a 24-hour period in order to collect 24 hour urine samples for 5-hydroxy indole acetic acid (5HIAA) excretion before and after use of BAS. Studies will be repeated in the non smokers who are able to smoke as many cigarettes as feasible for 72 hours building up to 30 cigarettes or more a day and repeating the use of BAS and the study. Similarly if feasible, the reverse study of 5HIAA excretion in the chronic smoker before and after cessation of smoking (the after representing no smoking in any form for at least 2 weeks). Pending completion of studies upon BAS it is intended to repeat similar observations with the use of weak chlorpromazine and other phenothiazine derivatives.

1003537270

6. Budget Plan:

Salaries	\$1,230
Expendable Supplies	2,275
Permanent Equipment	763
Overhead	640
Other	154
Total	4,910

7. Anticipated Duration of Work:

One year January 1, 1959 to December 31, 1959

8. Facilities and Staff Available:

General facilities of a 236 bed medical-surgical hospital with pulmonary function laboratory capable of carrying out analysis of pulmonary function involving lung volumes, mechanics of respiration, bronchospirometry, alveolar ventilation, blood gas analysis, pH determinations, fluoroscopy of the heart and chest and immediately adjacent electroencephalographic equipment.

5 physicians

3 full-time (1 laboratory director 2 research fellows)
2 part-time (1 cardiologist 1 chest physician)

9. Additional Requirements Senior technician (10 years experience as cardiopulmonary technician)

None

10. Additional Information (Including relation of work to other projects and other sources of supply):

(includes preliminary studies and work now in progress) Tobacco has been said to "tranquillize the spirit" (1). Theoretical considerations regarding this well recognized action of smoking tobacco in producing a certain amount of tranquilization or relief of tension and anxiety led us to the hypothesis that this action may be similar to the tranquilization produced by certain drugs like reserpine. It is currently generally accepted that this reserpine effect is associated with the release of serotonin in certain areas of the brain (2, 3). It has therefore been postulated by us that nicotine may likewise act on the central nervous system by the liberation of serotonin. It has been well established that nicotine has an important effect upon the autonomic ganglion cells. These effects in general consist first in stimulation and later in depression of these cells. There is some evidence that serotonin is involved in the transmission of the nerve impulse at this level (4). However, to our knowledge, no studies involving the use of nicotine in humans or of serotonin antagonists to study this action have been reported. If our hypothesis regarding the action of nicotine on the nervous system is correct, we should be able to block some of the important pharmacological effects of this agent by the use of antiserotonin substances. (Continued on page 2a).

Signature

Dr. R. E. Wells, Jr.

/s/ F. Lloyd Musella
Business Officer of the Institution

1003537271

We have chosen to begin our investigation of this possible action by utilizing BAS (benzyl analog of serotonin), a chemical compound synthesized by Woolley (5) for the specific purpose of blocking serotonin action.

Preliminary Observations: Nicotine has been administered intravenously to a series of normal human subjects, including smokers and non-smokers. Measurement of respiratory rate, minute volume, pulmonary airflow resistance, and compliance, blood pressure, electrocardiograms and in selected cases electroencephalograms have been recorded. The subjects have also been observed clinically for evidence of pallor, sweating, or syncope. Their subjective reactions have also been recorded. BAS has been administered intramuscularly to these subjects after these observations and before and after the intravenous administration of nicotine. It has been consistently noted in the cases studied thus far that this serotonin blocking agent also appears to block some of the important pharmacologic and physiologic effects of nicotine on the nervous and cardiovascular systems. Similar observations have been begun on previously studied patients with pulmonary emphysema and bronchial asthma who are known to have consistently predictable reactions from cigarette smoking, by administering BAS after cigarette smoking and noting the changes in the parameters described above when this antiserotonin agent is administered.

Further Studies: In relation to the above studies, an attempt is being made to determine whether the administration of nicotine intravenously results in an increase in the urinary excretion of 5-hydroxy indole acetic acid (5HIAA). Such an increase might be expected if nicotine causes the release of significant amounts of serotonin in the body. Along the same line of thought, the 24 hour excretion of 5HIAA is also being studied in groups of non smokers and smokers, including normal individuals and patients with broncho-pulmonary disease. (See cost of expendable supplies - cost of 5HIAA and other chemical analyses).

It is believed that these observations may yield information which will be of considerable interest from the point of view of a clearer understanding of the basic mode of action of nicotine in the body as a pharmacologic agent. It may also provide some avenues of approach for practical applications involving the use of nicotine blocking agents which might be found to counteract some of the undesirable pharmacologic effects of nicotine, while at the same time preserving the pleasure giving effect of tobacco smoking. In patients with certain types of cardiac or pulmonary disease who find it impossible to give up the smoking habit in spite of its effects upon them, some of the drugs which these studies may find effective might be important therapeutically.

The majority of equipment used in these studies has been purchased for studies of the mechanics of respiration by other research grants. The laboratory program which is involved in the study of respiratory mechanics in general has been supported by the Howard Hughes Medical Institute and the Massachusetts Heart Association. The specific study now underway most closely related to that proposed in this request concerns the effect of cold air inhalation upon the function of the heart and respiratory system.

1003537272

Bibliography

1. Krantz, J.C. and Carr, C.J.: The Pharmacologic Principles of Medical Practice. Williams and Wilkins, Baltimore, 1949.
2. Shore, P.A., Silver, S.L., and Brodie, B.B.: Interaction of Lysergic Acid Diethylamide (LSD) in the Central Nervous System. Experimentation 11: 272, 1955.
3. Shore, P.A., Silver, S.L., and Brodie, B.B.: Interaction of Reserpine, Serotonin, and Lysergic Acid Diethylamide in Brain. Science. 122:284, 1955.
4. Himwich, H.E.: Psychopharmacologic Drugs. Science. 127: 59, 1958.
5. Shaw, E.N., and Woolley, D.W.: Methylserotonins as Potent Antimetabolites of Serotonin Active both in vitro and in vivo. J. Pharmacol. Expt. Therapy, 116:164, 1955.
6. Costa, E.: The Effects of Hallucinogenic and Tranquilizing Drugs on the Serotonin Evoked Uterine Contractions. Psychiat. Research Rept. 4:11, 1956.

1003537273

Application For Research Grant

Date: **January 3, 1958**

1. Name of Investigator: **Roe B. Wells, Jr., M.D.**
2. Title: **Associate in Medicine, Peter Bent Brigham Hospital;
Clinic Associate in Medicine, Harvard Medical School;
Director of Chest Clinic and Pulmonary Function Laboratory,
Peter Bent Brigham Hospital.**
3. Institution
& Address: **Peter Bent Brigham Hospital
721 Huntington Avenue
Boston 15, Massachusetts**
4. Project or Subject: **A Study of the Correlation of Smoking History and Habits with
Pulmonary Function and the Acute Effects of Smoking Thereon in Patients with
Bronchitis, Bronchial Asthma, and the Common Cold with Similar Studies in a
Selected Geriatric Population of Apparent Good Health.**

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Rationale

This laboratory has recently completed studies of the effects of smoking upon pulmonary function. The majority of these patients were individuals with advanced chronic pulmonary disease. It is felt that a similar analysis is needed in the more common or reversible respiratory ailments such as bronchitis, bronchial (not cardiac) asthma and the common cold, since it is in these individuals that the physician lacks sufficient evidence regarding the role played by cigarette smoking. Such patients represent the major work load of this laboratory and its associated Chest Clinic.

As part of renewed interest in the process of aging, there is a well established geriatric clinic where an intensive study of the older members of the population is being carried on. This study includes studies of the respiratory system.

The facilities of the pulmonary function laboratory and the geriatric clinic afford rich opportunity to correlate smoking history and habits, and acute respiratory disease with pulmonary function.

Method of Study

All patients to be studied will have a complete medical history, physical examination, and routine laboratory procedures including blood count, urinalysis, chest-x-ray, and electrocardiogram. (Continued on page 1 a.)

1003537274

continued

A detailed history of smoking habits will be recorded on a protocol sheet prior to testing. This will include the total duration of smoking or chewing of tobacco of any kind, periods of interruption, relationship to time of day, prior illnesses, activities or stress and individual method of smoking, i.e., what portion is discarded, what holders or filters may be used and under what environmental conditions is the smoke inspired. (We have found it of value to corroborate or amplify these details with at least one member of the patient's family in a separate interview.) Finally the patient's attitude toward his smoking is explored. He is asked why he smokes, why he started, has he tried to stop, and has his opinion about smoking ever changed.

Patients studied will be in a comfortable seated position, usually in the fasting state, specifically not earlier than two hours after the last meal. The standard lung volumes are recorded on a closed circuit high speed spirometer and when appropriate, include determination of residual volume by the closed circuit helium method. Following this, the intra-esophageal balloon technique is used to measure mean pulmonary airway resistance and pulmonary compliance. This is also done with a closed circuit system, recording tidal volume by way of a rotational potentiometer attached to the spirometer and led into a recording galvanometer. On the same time axis is the recording from a transducer of the intra-esophageal pressure (adapted from the original method of Mead and Whittenberger). If the clinical history, examination, or routine laboratory data suggest any cause for arterial oxyhemoglobin unsaturation, arterial blood samples will be obtained from an indwelling arterial needle (brachial or radial).

Following these baseline studies (roughly 10 - 15 minutes excluding the residual volume) the patient smokes one cigarette in the manner in which he is accustomed. The method, amount, and duration of smoking are noted and recorded. The above procedure of mean airway resistance determination is repeated at once and again in 7 to 8 minutes after cessation of smoking. With an oscilloscope wired from the recording galvanometers changes in airway resistance are noted at once. On the basis of these observations the cigarette smoking is repeated once again (within 10 minutes) or a nebulized bronchodilator (Isoproterenol) administered. If this latter agent is effective, its preventive abilities are evaluated by repeating the cigarette smoking at various intervals up to 45 minutes. (The various times reported here (7 to 8 minutes, 45 minutes, etc.) are derived from prior experience in this laboratory as the most comprehensive periods for recording the various effects).

Studies in the geriatric study group will follow the same pattern. The other studies of these patients mentioned above will be integrated when appropriate with the data on smoking.

1003537275

1st year.	End yr.		3rd yr.	Total 3 yrs.
6. Budget Plan:				
\$2,000.00	\$2,000.00	Salaries	\$2,000.00	\$6,000
750.00	750.00	Expendable Supplies	750.00	2,250
4,760.00	850.00	Permanent Equipment		5,610
1,877.50	900.00	Overhead 25%	687.50	3,465
		Other		
\$9,387.50	\$4,500.00		\$3,437.50 Total	17,325

* As prescribed by the Trustees of the Institution.

7. Anticipated Duration of Work: Three years (July 1, 1958 to June 30, 1961)

8. Facilities and Staff Available: Pulmonary Function Laboratory of a 286 medical-surgical bed hospital. The laboratory is equipped to carry out determinations of all lung volumes, mechanical factors of respiration, blood and respiratory gas analyses and their tensions, pH, intra-vascular and thoracic pressures, as well as the various compartments and functions derived from these, such as respiratory dead space, alveolar arterial oxygen gradients and respiratory responses to inhalation of various gas mixtures. Fluoroscopy and x-ray facilities are immediately adjacent. (Continued on page 2 a.)

9. Additional Requirements: None

10. Additional Information (Including relation of work to other projects and other sources of supply):

The relation of this proposed study to other work is answered in part under the protocol of item 5 above. Besides the geriatric study, the other study now in progress in this laboratory and also referred to above involves the study of sputum viscosity, primarily in relation to high humidity therapy (steam or fog) and the effect of various agents upon sputum production and character.

In regard to other projects outside the hospital we have followed with interest the reports from Dr. J. Howland Auchincloss of Syracuse and through personal communication the work of Dr. Ernest Attinger in this related field. Concerning the basic methodology there is constant liaison with the Department of Physiology of the Harvard School of Public Health wherein excellent work in the general field of the mechanics of respiration is being carried out. (This group has studied 5 smokers over the age of 50 and found an elevated airway resistance in 4.) (Continued on page 2 a.)

Signature /s/ Roy E. Wells
Director of Project

/s/ Victoria Caas M. M.D.

Business Officer of the Institution

1003537276

continued

5 physicians

3 full-time (1 laboratory director 2 research fellows)
2 part-time (1 cardiologist 1 chest physician)

Active cooperation and collaboration with members of the Thoracic Service.

1 senior technician (10 years experience as cardiopulmonary technician)

1 technician-secretary

10. continued

For 1958-1961 there are no other sources of supply regarding the laboratory other than the very small remainder (less than \$300) of the funds that were provided to establish the laboratory some time ago. The laboratory director is supported by the Howard Hughes Medical Institute.

1003537277

RECEIVED

MAY 29 1959

PHILIP MORRIS & CO. LTD. INC.
RESEARCH & DEVELOPMENT
DEPT.

1003537278



THE UNIVERSITY OF OKLAHOMA

NORMAN · OKLAHOMA

December 7, 1955

T.H.C. - Wender
Applied Chem.
DEPARTMENT OF
CHEMISTRY

Dr. Robert N. DuPuis
Vice President - Research
Philip Morris, Inc.
P. O. Box 1895
Richmond 15, Virginia

Dear Dr. DuPuis:

My visit with you was indeed a pleasant and educational experience. And as I told you last Saturday, you have organized and inspired a young, enthusiastic research team at Philip Morris that will, I am confident, make major contributions in tobacco research.

I sincerely thank you for your hospitality, and I trust that we may again meet in the future.

Sincerely,

Simon H. Wender
Simon H. Wender

SHW:ns

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THE NEW AMERICAN CRYSTAL COMPANY

42-3

195

From the desk of:

ROBERT N. DU PUIS

File
a

(c):

Enjoyed visit & perzited Priefron.
Principal question was which
type of tobacco to use.
Will handle thru Bob Hockett.
May want to come again. This
was encouraged. Perhaps
should visit Reynolds also.

1003537281

November 28, 1955

Dr. Simon H. Wender
The University of Oklahoma
Norman, Oklahoma

Dear Dr. Wender:

We are happy to learn that you will definitely be with us Friday, December 2 and Saturday morning, December 3. We are suggesting that you spend Friday morning in this laboratory and Friday afternoon at the laboratory of the American Tobacco Company. At that point, we can decide how best to make Saturday morning as productive as possible in the matter of discussing our mutual problems.

I suggest that you come to our laboratory at 7th and Stockton Streets about nine o'clock Friday morning, December 2.

We are looking forward to seeing you at that time.

Sincerely,

Robert N. DuPuis
Vice President - Research

RND:BS
Airmail

cc: Mr. H. R. Hanmer
American Tobacco Co., Inc.
Research Laboratory
400 Petersburg Pike
Richmond, Virginia

1003537282



THE UNIVERSITY OF OKLAHOMA

NORMAN · OKLAHOMA

November 21, 1955

*Here Fri am.
Joint lunch ATCo Fri
@ ATCo Fri p.m.
Then decide Sat. program*

Dr. Robert N. DuPuis
Vice-President - Research
Philip Morris Incorporated
P. O. Box 1895
Richmond 15, Virginia

Dear Dr. DuPuis:

I am happy to confirm Friday, December 2 as the date of my visit to Richmond. I plan to arrive in Richmond by Thursday evening, and will arrange to stay until Saturday afternoon. This will enable me to have the entire day Friday and also all of Saturday morning with you and your committee. I trust that these plans will be satisfactory to you all.

Sincerely yours,

Simon H. Wender
Simon H. Wender

S HW:ns

1003537283

RECEIVED

NOV 23 1935
PHILIP MORRIS & CO. LTD. INC.
RESEARCH & DEVELOPMENT
DEPT.

1003537284

TIRC - *Re: Application*

November 9, 1955

Dr. Simon H. Wender
Research Professor of Chemistry
University of Oklahoma Research Institute
Norman, Oklahoma

Dear Dr. Wender:

Thank you very much for your letter of October 31. We would be very happy to meet with you in Richmond and your suggested date of Friday, December 2, is quite satisfactory with us. I have discussed the matter with Mr. H. R. Hanmer, Director of Research of the American Tobacco Company in Richmond and he also would be happy to meet with you on that date.

I would suggest that you plan to spend at least a half day in the American Tobacco Company laboratory and another half day with us. If your travel schedule permits, I think that an additional half day for general discussion might also be advisable, making a total of a day and a half.

We shall be very happy to have your confirmation of the date of your visit. We are all looking forward to meeting you.

Sincerely,

Robert N. DuPuis
Vice President - Research

RND:BS

cc: Mr. H. R. Hanmer
Dr. Robert C. Hockett
Mr. A. E. O'Keeffe

1003537285



DEPARTMENT OF
CHEMISTRY

THE UNIVERSITY OF OKLAHOMA

NORMAN · OKLAHOMA

October 31, 1955

Howard Wright
Raeburn
HPH
will
all date
OK EATCO
CPH DPH

Dr. Robert N. DuPuis
Vice President in Charge of Research
Philip Morris, Inc.
Box 1859
Richmond, Virginia

Dear Dr. DuPuis:

As you know, Dr. Robert C. Hockett, Associate Scientific Director, Tobacco Industry Research Committee, has written me concerning the advantages of a visit by me to Richmond to meet with you and certain members of the Industry Technical Committee. I believe that this is an excellent idea, and I am anxious to avail myself of this opportunity.

Would one full day in Richmond be sufficient for this meeting or would it be better to plan for a more extended visit? What dates would be most suitable for me to visit your Committee in Richmond? At the moment, Friday, December 2, 1955 appears to me to be one of several likely prospective dates for a visit.

OK here
ck HPH

Sincerely yours,

Simon H. Wender
Simon H. Wender

S HW:ns

1003537286

2-10-1944

STANDARD OIL COMPANY

STANDARD OIL COMPANY

THE STANDARD OIL COMPANY, NEW YORK, N.Y., HAS THE HONOR TO ACKNOWLEDGE THE RECEIPT OF YOUR LETTER OF THE 29TH INSTANT, IN WHICH YOU REQUESTED THAT WE ADVISE YOU OF THE RESULTS OF OUR INVESTIGATION INTO THE MATTER OF THE ALLEGED VIOLATION OF THE FEDERAL TRADE COMMISSION ACT BY THE STANDARD OIL COMPANY, NEW YORK, N.Y., IN CONNECTION WITH THE SALE OF KEROSENE FUEL TO THE UNITED STATES ARMY AND NAVY.

IT IS EXTREMELY DIFFICULT TO OBTAIN THE INFORMATION REQUESTED BY YOU, AND WE ARE SORRY THAT WE ARE UNABLE TO FURNISH IT TO YOU AT THIS TIME. WE ARE, HOWEVER, CURRENTLY CONDUCTING AN INVESTIGATION INTO THE MATTER, AND WE WILL ADVISE YOU OF THE RESULTS OF OUR INVESTIGATION AS SOON AS THEY ARE AVAILABLE.

Yours very truly,

J. EDWARD HENNING

FOR 1944

STANDARD OIL COMPANY, NEW YORK, N.Y.

STANDARD OIL COMPANY, NEW YORK, N.Y.

STANDARD OIL COMPANY, NEW YORK, N.Y.

RECEIVED
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STANDARD OIL COMPANY
NEW YORK, N.Y.

STANDARD OIL COMPANY

STANDARD OIL COMPANY

1003537287

TIRC - Res. Grants

W.H.C.
10/21/55

File

October 21, 1955

Dr. Simon H. Wender
Research Professor of Chemistry
University of Oklahoma Research Institute
Norman, Oklahoma

Dear Dr. Wender

The notification that your application for a grant-in-aid to study the polyphenols in tobacco and cigarette smoke has been granted is being sent today by our Executive Secretary. We are pleased that this support is possible and look forward to a mutually beneficial association.

I think it will be appropriate for me to comment further without delay upon several points that were discussed by the Scientific Advisory Board during the consideration of your proposal.

The income of the Tobacco Industry Research Committee is received in a manner which makes it unwise to enter into any firm or legal commitment for more than one year at a time. At the same time, the members of the Scientific Advisory Board are well aware of the need for continuity in research of many kinds. Their policy is to recognize the need for such continuity over several years by making a grant for one year only but undertaking to give prior consideration to the needs of such projects in succeeding years over new applications, providing only that satisfactory progress is being made in the light of all the usual hazards and uncertainties of any research.

Such priority of consideration can be maintained for a period of two or three years. So far the Board has not, in any case, undertaken to promise it for as long a period as five years.

When the period of such prior consideration has expired, any recipient of a grant is still at liberty to submit a new proposal for consideration in competition with all other proposals in hand, and in the light of the situation that may then exist.

1003537288

Dr. Simon H. Wender

-2-

October 21, 1955

I hope that this statement will clarify the policies of the Board as they relate to your studies.

The other matter which the Board wishes to bring to your attention is the fact that there is a very considerable accumulation of analytical technique and information within the laboratories of the tobacco industry. An Industry Technical Committee, comprising research directors of the several companies, has been constituted to advise the Scientific Advisory Board in matters bearing upon tobacco technology. The Board members feel that it might be very advantageous for you to visit certain members of this committee in order to exchange information and ideas in the interest of saving time in experiment and avoiding any unnecessary duplications of effort.

If you agree, as we hope you will, that this might provide valuable and stimulating contacts, the necessary arrangements can be made by communicating directly with the chairman, Dr. Robert N. DuPuis, Vice President in Charge of Research, Philip Morris, Inc., Box 1839, Richmond, Virginia. Dr. DuPuis has agreed to arrange for you to visit him and certain other members of the Industry Technical Committee at your mutual convenience.

It is the sense of the Board that it will be appropriate for you to use travel funds provided in the grant for a trip to Richmond for this purpose, and they are prepared, if necessary, to supplement the travel allowance in order to bring about these conferences effectively and at an early date.

Very sincerely yours,

R. C. H.

Robert C. Hockett
Associate Scientific Director

RCH:mg

cc: ✓ Dr. Robert N. DuPuis

1003537289

1003537290

Dr. Simon H. Wender

October 21, 1952

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Very sincerely yours,

Robert C. Hockett
Associate Scientific Director

cc: Dr. Robert W. DuPont

Application For Research Grant

OK
Date: August 22, 1955

Date:

1. Name of Investigator: **Dr. Simon H. Wender**
2. Title: **Research Professor of Chemistry**
3. Institution & Address: **University of Oklahoma Research Institute
Office of the Executive Director
Norman, Oklahoma**
- would like to confer*

4. Project or Subject: **A qualitative and quantitative study of the individual polyphenol content of cigarette tobacco and of the smoke and "tars" resulting from cigarette smoking, and also to study the fate of these compounds in the animal respiratory system.**

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

See #10 for background information

The proposed research will involve:

(a) expanded qualitative and new quantitative studies of as many as possible of the individual polyphenolic compounds present in tobacco leaves;

(b) the actual isolation of as many as possible of pure, randomly labeled, polyphenolic compounds from radioactive tobacco (now available from Argonne National Laboratory);

(c) qualitative and quantitative studies of the individual polyphenols present in the tobacco of commercial cigarettes and then

(d) using individual, radioactive polyphenolic compounds known to be generally present in the cigarette (in non-radioactive condition, of course), in studies of the fate of these compounds in the smoke and "tars" resulting from the smoking of cigarettes; and

(e) studies on the fate of these compounds in the respiratory system of laboratory test animals.

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8. Facilities and Staff Available -- continued

Dr. Wender was born in Dalton, Georgia, September 4, 1913. He received the A.B. and M.S. degrees at Emory University, Georgia, in 1934 and 1935, respectively, and the Ph.D. in Agricultural Biochemistry (Major Professor, the late Dr. Ross A. Gortner) from the University of Minnesota in 1938. Member, Phi Beta Kappa, Sigma Xi, Phi Lambda Upsilon, Phi Sigma, ~~Sigma~~ Alpha Chi Sigma, American Chemical Society, (now chairman of its Oklahoma section) American Society of Biological Chemists, American Association for the Advancement of Science, Society for Experimental Biology and Medicine, Chemists Club of Central Oklahoma, and Oklahoma Academy of Science (Fellow).

Dr. Wender is representative of the University of Oklahoma to the Council of the Oak Ridge Institute of Nuclear Studies, and is a consultant to the Division of Biology and Medicine, Argonne National Laboratory.

Plant Physiologist, Dr. Lawrence M. Rohrbaugh, Professor of Plant Physiology at the University of Oklahoma, will be the consultant on plant physiology phases of the research.

Dr. Marvin Shetlar, Chief of the Medical Research Laboratory, V. A. Hospital, Oklahoma City, will cooperate on ~~the~~ biochemical and physiological studies and act as a consultant to the project.

Other consultants on many specialties, including cancer specialists, will be available as necessary, at the School of Medicine, University of Oklahoma.

10. Additional Information -- continued

these polyphenols may be of some import in the planned analyses of tobacco smoke.

Reprints, where available, of some of the above and other pertinent articles are attached, as is a list of publications ~~of~~ Dr. Wender.

During the summer of 1954, while Dr. Wender was a research participant working with Dr. Norbet J. Scully and his staff at the Argonne National Laboratory, studies aimed at separating and identifying the many, individual polyphenolic compounds present in tobacco leaves were undertaken. Considerable initial progress was made, both on regular tobacco and also on tobacco plants grown in an atmosphere containing Cl^{40}_2 . This research has as its main purpose the ~~acquire~~ acquisition of fundamental knowledge about the composition and metabolism of polyphenolic compounds in ~~plants~~ plants, and is still in active progress, with support from the U. S. ~~Atomic~~ Atomic Energy Commission.

During the past year, Dr. Wender and students at Oklahoma have further improved their chromatographic techniques, and have now successfully obtained more than 20 pure compounds from tobacco. No doubt, several of these compounds will be found to be identical on ultimate analysis, and two of these compounds have been previously reported to be present in tobacco. It appears, however, that at least 15 of these polyphenolic compounds are individuals not previously reported in tobacco. Fine progress is now being made on the difficult task of identification of these compounds.

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These isolation methods and this knowledge are now available for application to the tobacco grown in an atmosphere of $Cl^{14}O_2$ at the Argonne National Laboratory. Their application would make available many individual ~~xx~~ radioactive polyphenols for further studies.

A chemical method for the preparation of specifically labeled quercetin ~~xxxxxx~~ (one of the compounds present in tobacco) is now being worked out at Oklahoma, and the Oklahoma group is now well along in the actual synthesis of this radioactive polyphenol. When completed, this will further supplement the potent array of tools for a possible investigation of the subject.

None of this work of the past year has as yet been published, but some of the isolation and identification studies are outlined in two M.S. theses just filed this month at the University of Oklahoma (M.S. theses of Mr. Edwin L. Murphy and Mr. Chao-Hsu Hwa Yang).

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Publications by Dr. Simon H. Mender

"The Isolation of Photosensitizing Agents from Buckwheat," J. Am. Chem. Soc. 65 1733 (1943).

"Preliminary Studies on the Separation of Ether-Insoluble Pigments from Tobacco," Trans. Ky. Acad. Sci. 11 36 (1943)

"Studies on Toxic Substances of Locoweeds," Texas Agric. Exp. Sta. Bull. No. 650 (1944).

"The Action of Photosensitizing Agents from Buckwheat," Amer. J. Vet. Res. 3 486 (1946)

"Studies on Flavone-Like Substances Isolated from Tobacco," Trans. Ky. Acad. Res Sci. 12 10 (1947)

"A Method for the Separation of Certain Flavones Present in Tobacco," Proc. Okla. Acad. Sci. 27 95 (1947)

"The Use of Amberlite Resin in a Separation of Xanthine from Guanine," J. Am. Chem. Soc. ~~70~~ ⁷⁰ 3719 (1948)

"The Ultra³ violet Absorption Spectrum of Waringin by Use of the Beckman Model DU Spectrophotometer," Proc. Okla. Acad. Sci. 29 57 (1948)

"The Purification and Quantitative Estimation of Quercetin by Paper Partition Chromatography," Proc. Okla. Acad. Sci. 29 64 (1948)

"Chromatographic Adsorption Studies on Certain Flavones," Proc. Okla. Acad. Sci. 29 71 (1948)

X "Asparagine Content of Burley Tobacco After Long Storage," Bot. Gaz. 110 637 (1949)

"Paper Chromatography of Flavonoid Pigments," Science 109 287 (1949)

"Paper Chromatography of Flavonoid Pigments: II Separation and Quantitative Estimation of Rutin and Quercetin," Federation Proc. 8 298³ (1949)

X indicates that reprints are on file in the Tobacco Industry Research Committee office.

1003537294

- X "The Isolation of Isoquercitrin from the Seed Pods of Cercia Canadensis,"
J. Am. Chem. Soc. 71 2658 (1949)
- "Preliminary Studies on the Use of Amberlite Resins in Locoweed Investigations," Proc. Okla. Acad. Sci. 30 130 (1949)/
- "The Use of Metal Complexes in Identification of Flavonoid Pigments,"
Proc. Okla. Acad. Sci. 30 145 (1949)
- "A Method for the Purification of Ethanol for Use in Ultraviolet Absorption Spectrophotometry," Proc. Okla. Acad. Sci. 30 149 (1949)
- "The Calibration of Pipettes for Paper Partition Chromatography," Proc. Okla. Acad. Sci. 30 144 (1949)
- "Use of Multiple Strips in One-Dimensional Paper Chromatography," Proc. Okla. Acad. Sci. 30 153 (1949)
- X "The Isolation of Isoquercitrin from Air-Dried Tobacco," Arch. Biochem. 22 74 (1950)
- "A Simplified Laboratory Experiment in Paper Partition Chromatography,"
J. Chem. Educ. 27 159 (1950)
- X ~~"The Quantitative Determination of Certain Flavonol-3-Glycoside~~
by Paper Partition Chromatography," Analytical Chem. 22 709 (1950)
- X "The Isolation of Quercitrin from Peanut Hulls," J. Am. Chem. Soc. 72 4177 (1950)
- "The Isolation of a Flavonoid Glycoside from Gleditsia triacanthos,"
Proc. Okla. Acad. Sci. 31 137 (1950)
- "Preliminary Studies on a Flavonoid Pigment from the Fruit of Plantanus occidentalis," Proc. Okla. Acad. Sci. 31 138 (1950)
- "The Use of Ion Exchange Resins in the Isolation of Flavonoid Compounds from Okra," Proc. Okla. Acad. Sci. 31 140 (1950)

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"Preliminary Investigations on the Flavonoid Pigments of Prunes," Proc.

Okla. Acad. Sci. 31 93 (1950)

"Chemical Studies on a Toxic Concentrate from the Big Bend Locoweed,"

Proc. Okla. Acad. Sci. 31 66 (1950)

"Methods for the Isolation of Quercitrin from Peanut Hulls," U. S. Patent

2,557,164 (1951)

X "The Use of Ion Exchange Resins in the Isolation of Flavonoid Compounds,"

Science 113 522 (1951)

X "The Use of N-bromosuccinimide and Pyridinium Bromide Perbromide for the

Conversion of Flav⁹anones into Flavones," J. Org. Chem. 16 930 (1951)

X "The Isolation of Morin on an Ion Exchange Resin," J. Am. Chem. Soc.

73 3340 (1951)

X "The 2,4-Dinitrophenylhydrazine Derivatives of Some Flavanones," J. Amer.

Chem. Soc. 73 4023 (1951)

"Identification of Flavonoid Compounds by Filter Paper Chromatography,"

Anal. Chem. 23 1582 (1951)

"The Use of Filter Paper Pulp in the Separation of Certain Flavonoid

Compounds," Proc. Okla. Acad. Sci. 32 99 (1951)

"Preliminary Studies on the Anthocyanins of *Phytolacca americana* on

Amberlite IRC-50," Proc. Okla. Acad. Sci. 32 101 (1951)

X "Quantitative Paper Chromatography Studies on Flavonol Aglycones," Proc.

Okla. Acad. Sci. 32 102 (1951)

"The Use of Ion Exchange Resins in the Decomposition of Lead Salts of

Flavonoid Compounds," Proc. Okla. Acad. Sci. 32 100 (1951)

"A Convenient Laboratory Model Wet Grinder and Extractor," Anal. Chem.

24 767 (1952)

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- X "Acid Dissociation Exponents of Rutin and Xanthohummin," J. Am. Chem. Soc. 74 143 (1952)
- X "A New Method for the Isolation of Pure Quercitrin from Lemon Flavin," Arch. Biochem. and Biophys. 32 195 (1952)
- X "The Isolation and Identification of Quercetin and Isoquercitrin from Grapes (Vitis vinifera)," J. Amer. Chem. Soc. 74 4372 (1952)
- X "The Isolation and Identification of Quercetin and Isoquercitrin from Black Currants (Ribes nigrum)," J. Amer. Chem. Soc. 74 4566 (1952)
- X "The Synthesis of Isoquercitrin," J. Amer. Chem. Soc. 74 4606 (1952)
- X "Adsorption Chromatography of Flavonoid Compounds," Anal. Chem. 24 1616 (1952)
- X "The Isolation and Identification of Kaempferol and Quercetin from Strawberries (Fragaria chiloensis)," J. Amer. Chem. Soc. 74 5919 (1952)
- X "Studies on Flavonoids of Locoweed," Proc. Okla. Acad. Science 33 251 (1952)
- X "Further Fractionation of a Toxic Concentrate from Big Bend Locoweed," Proc. Okla. Acad. Science 33 253 (1952)
- X "The Isolation of a Flavonoid Substance from Watermelon," Proc. Okla. Acad. Science 33 247 2 (1952)
- X "Isoquercitrin and Quercetin in Concord Grapes," Proc. Okla. Acad. Science, 33 250 (1952)
- X "Preliminary Investigations of the Possible Flavonol Content of the Pineapple," Proc. Okla. Acad. Science 33 249 (1952)
- X "Identification of Flavonoid Compounds by Filter Paper Chromatography. II Additional R_f Values and Color Tests," Anal. Chem. 25 508 (1953)
- X "Quercetin and Its Glycosides in Leaves of Vaccinium myrtillus," J. American Chem. Soc. 75 50 (1953)

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- X "Isolation and Identification of Quercetin and Isoquercitrin from Apricots (Prunus armeniaca)" Arch. Biochem. and Biophys. 43 22 379 (1953)
- X "An Improved Method of Analysis for Glycine Using Streptococcus Faecalis A.T.C.C. 6057," Arch. Biochem. and Biophys. 43 485 (1953)
- X "Hydrolysis of Some Flavonoid Rhamnoglucosides to Flavonoid Glucosides," J. Amer. Chem. Soc. 75 2504 (1953)
- "Some Factors Affecting the Nutritional Requirements of Streptococcus Faecalis A.T.C.C. 6057," J. Bact. 65 660 (1953)
- "Separation and Determination of Pyridoxine, Pyridoxal, and Pyridoxamine by Paper Chromatography and Microbiological Assay," Arch. Biochem. and Biophys. 45 465 (1953)
- X "Isolation and Identification of Quercetin and Some Quercetin Glycosides from Plums (Prunus salicina)," J. Amer. Chem. Soc. 75 4363 (1953)
- X "Enzymatic Hydrolysis of Certain Flavonoid Glycosides," J. Amer. Chem. Soc. 76 1950 (1954)
- "Ion Exchange Studies on the Flavonoid Fraction from Licorice Root", Proc. Okla. Acad. Sci. (1953) 34 163 (1953)
- "Isolation of Flavonoid Compounds," U. S. Patent No. 2,681,097 (1954)
- X "Asperometric Titrations of Some Flavonoid Compounds with ~~Rare~~ Cupric Sulfate," J. Am. Chem. Soc. 77 162 (1955)
- Patents Pending through the Atomic Energy Commission
- "Separation of Flavonoid Compounds," A.E.C. Case No. S-4300 (S. N. 285,046)
- "Improved Method for the Separation of Flavonoid Compounds," A.E.C. Case S-10,133 (S. N. 323,505)
- "Synthesis of an Aldohexoside of a Flavonol," A.E.C. Case S-10,134 (S. N. 339,928)
- "A Method for the Conversion of Flavonoid Glucosides," A.E.C. Case No. S-10,724

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6. Budget Plan:

Salaries	\$ 5,250.00
Expendable Supplies	1,250.00
Permanent Equipment	1,000.00
Overhead	1,170.00
Other (travel, transp., com.)	300.00
Total	\$ 8,970.00

7. Anticipated Duration of Work: It is estimated that some three to five years will be necessary for satisfactory achievement of the major objectives of the projected research. Considerable progress, however, could be expected within one year.

8. Facilities and Staff Available:

Adequate research facilities for the proper prosecution of the investigation are available.

Staff available: Plant Biochemist, Dr. Simon H. Wender

The project will be supervised by Dr. Simon H. Wender, Research Professor of Chemistry, Department of Chemistry, University of Oklahoma (see attached sheet)

9. Additional Requirements:

None

10. Additional Information (Including relation of work to other projects and other sources of supply):

In the past few years, by means of chromatographic adsorption, paper chromatographic, and ion exchange methods recently adapted to the polyphenolic flavonoid compounds in the laboratories of the University of Oklahoma, it has been found possible to effect the separation of flavonoid compounds from the tannins, anthocyanins, and other phenolic compounds present in the plant extracts and also to separate individual flavonoids from each other. (Anal. Chem. 22 709 (1950); 23 1582 (1951); 24 1616 (1952); and 25 508 (1953); Science 109 287 (1949) and 113 522 (1951). In addition, paper chromatography may be used for the qualitative and quantitative analyses of individual compounds in this group. By such techniques, Oklahoma ~~WER~~ workers have been able to identify the polyphenolic flavonoid compound isoquercitrin in tobacco which had been stored for more than seven years (Arch. Biochem. 25 74 (1950). Work with anthocyanins and other phenolic compounds has indicated similar possibilities with them. Thus, chromatography, together with the extremely sensitive tool of radioactive polyphenols, now make the research proposed here feasible. Also many of these compounds to be studied have high melting points and readily sublime. Therefore, (continued on attached sheet)

s/ Simon H. Wender

Signature _____
Director of Project

1003537299

s/ Lloyd E. Swearingen

Business Officer of the Institution

Executive Director - University of
Oklahoma Research Institute, Norman, Oklahoma

Scopoletin in Commercial Tobacco Products

Chao-Hwa Yang, Yasushi Nakagawa and Simon H. Wender

Chemistry Department, University of Oklahoma
Norman, Oklahoma, U.S.A.

Table 1.—Determination of scopoletin in tobacco

Tobacco product type and brand	Volume (ml) aliquot streaked on paper	Scopoletin (μ g) per aliquot	Scopoletin (μ g) per 1 g tobacco*	Scopoletin (%) in tobacco
(1)	(2)	(3)	(4)	(5)
Cigars				
1C				
Sample 1	10	18.4	92.0	0.0092
Sample 2	10	17.6	88.0	0.0088
2C				
Sample 1	10	19.3	96.5	0.0097
Sample 2	10	19.1	95.5	0.0096
3C				
Sample 1	10	5.8	29.0	0.0029
Sample 2	10	5.5	27.5	0.0028
4C				
Sample 1	10	7.3	36.5	0.0037
Sample 2	10	7.3	36.5	0.0037
Hand-rolled Cigarette Tobacco				
1HC				
Sample 1	15	26.1	87.0	0.0087
Sample 2	15	27.3	91.0	0.0091
2HC				
Sample 1	15	28.5	95.0	0.0095
Sample 2	15	28.0	93.3	0.0093
Pipe Mixtures				
1PM				
Sample 1	15	23.7	79.0	0.0079
Sample 2	15	23.5	78.3	0.0078
2PM				
Sample 1	15	26.0	86.7	0.0087
Sample 2	15	28.8	96.0	0.0096
3PM				
Sample 1	15	25.3	84.3	0.0084
Sample 2	15	24.4	81.3	0.0081
Pipe Tobaccos				
1P				
Sample 1	15	17.8	59.3	0.0059
Sample 2	15	18.7	62.3	0.0062
2P				
Sample 1	15	13.8	46.0	0.0046
Sample 2	15	14.4	48.0	0.0048
3P				
Sample 1	15	16.7	55.7	0.0056
Sample 2	15	14.0	46.7	0.0047

Following the discovery by Yang *et al.* (1958) that the tobacco and the mainstream smoke from cigarettes commonly used in the U. S. contained scopoletin (6-methoxy-7-hydroxycoumarin), these workers devised quantitative methods for its determination in cigarette tobacco and smoke. Recently, they reported quantitative results obtained for scopoletin in 24 brands of cigarettes (Yang *et al.*; in press). These findings have suggested the extension of qualitative and quantitative scopoletin studies to other commercial tobacco products. This paper reports the identification of scopoletin in all the cigars, snuff, chewing tobaccos, pipe tobaccos, pipe mixtures, and roll-your-own cigarette tobaccos analyzed. Quantitative results are reported on selected samples of each type of product as well as on the smoke from selected cigars.

Materials and Apparatus

Tobacco samples—Each tobacco product tested was purchased locally on the open, retail market. Cigars studied were El Producto (Puritano), Wm. Penn (Perfecto), Robt. Burns (de Luxe), Robt. Burns (Cigarillo), White Owl (Perfecto), Roi-Tan (Perfecto), Roi-Tan (Golfer), El Verso (Bouquet), El Verso (Mellow), King Edward, Melba, Melba (Midget), Webster (Babies), Corina (Magnolia), Corina (Larks), Corina (Cigarillo), Coronitas (Perfecto), Dutch Masters (Corona de Luxe), Dutch Masters (Crown), Dutch Masters (Belvedere), Dutch Masters (President), Muriel, Royalist (New Duke), Cuesta-Ray, Antonio y Cleopatra, El Trelles, Red Dot (Perfecto), Red Dot (Cigarillo), Mississippi River, Hunter (Imperial), and

* This research was supported, in part, by the Tobacco Industry Research Committee and the Atomic Energy Commission.

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Little Fendrich (Buds).

Pipe tobaccos and mixtures and hand-rolled cigarette tobaccos included Kite, Bugler, Revelation, Walnut, London Dock, Bond Street, Half and Half, George Washington, Edgeworth, Dill's Best, Model, Sir Walter Raleigh, Prince Albert, Velvet, Briggs, and Sutliff's Mixture No. 79. Snuffs tested were called Honest Scotch Snuff, Scotch Snuff, Sweet Mild Snuff, and Copenhagen Snuff. The chewing tobaccos studied were Bull of the Woods, Day's Work, W. N. Tinsley, Tinsley's Natural Leaf, Skoal Wintergreen Flavored, and Beech-Nut.

Chromatography paper and chambers—The chromatography paper used throughout these analyses has been Schleicher and Schuell No. 589, Red Ribbon. Purchased in 58x58 cm. sheets, the papers, unless otherwise noted, have been cut to a size approximately 19x58 cm. each for use. Descending chromatography in conventional 12"x24" Pyrex chromatography jars and in the standard, stainless steel interior chromatography cabinets (approximately 27½"x26"x-19½" inside dimensions) has been employed.

Smoking machine—To obtain cigar smoke for scopoletin analysis the cigar was smoked on a standard cigarette smoking apparatus (Phipps and Bird, Inc., Richmond, Va.) based on a design of the American Tobacco Company. The holder was modified to accommodate various cigar sizes. To trap the smoke, three Kjeldahl flasks were used in series. The first flask, 300 ml. capacity, was equipped with a spiral tube, one end of which was connected to the cigar holder and the other end of which reached the bottom of the flask. A small glass stopper was attached to the end of the side arm. During smoking, this stopper could be removed and solvent added from the side arm to wash down the smoke which condensed inside the spiral tube. Straight glass tubes were used for the second and third flasks, each of which flasks was of 100 ml. capacity.

Experimental

Preparation and extraction of sample—Each tobacco product was ground with a Wiley intermediate mill, 40 mesh screen. The samples were not dried beforehand, except for three brands of chewing tobacco, 4 CH, 5 CH, and 6 CH. These were dried at 50° for 48 hrs. prior to grinding. Approximately 4 g. of each of these ground tobacco powders were weighed and transferred into individual Soxhlet thimbles for ex-

traction.

Each 4 g. extraction was carried out in a separate Soxhlet extractor, using 250 ml. of 85 percent isopropyl alcohol for approximately 3 hrs. on a steam bath. A second extraction was made on each sample, again using 250 ml. of 85 percent isopropyl alcohol for 3 hrs. The two extracts of the 4 g. tobacco sample were combined, reduced to approximately 150 ml. in vacuo, and the volume was then adjusted with 85 percent isopropyl alcohol to 200 ml. in a volumetric flask. Aliquots of this solution were then taken for qualitative and for quantitative analyses of scopoletin.

Smoking procedure—Prior to smoking, 25 ml., 10 ml., and 10 ml. of an anhydrous acetone-absolute ethyl alcohol (1:1 v./v.) mixture

were placed in the first, second, and third flasks, respectively. The Kjeldahl flasks used as traps were then lowered into large evacuated flasks (modified Dewar) containing dry ice-acetone (approximately -77°) for at least 30 minutes before smoking and were kept in the cold baths throughout the smoking.

The puff duration, puff interval, and volume of smoke per puff were 3.5 seconds, 30 seconds, and 53-57 ml, respectively. Exact volume of smoke per puff, number of puffs, butt length, humidity and room temperature for the quantitative studies have been recorded in table 2.

After the cigar samples had been smoked, the trap system, still connected in series, was removed from the Dewar flasks, and was left until

Table 1—continued

	(1)	(2)	(3)	(4)	(5)
Pipe and Cigarette Tobaccos					
1PC					
Sample 1		15	11.6	38.7	0.0039
Sample 2		15	10.2	34.0	0.0034
2PC					
Sample 1		15	10.0	33.3	0.0033
Sample 2		15	10.4	34.7	0.0035
3PC					
Sample 1		15	10.5	35.0	0.0035
Sample 2		15	9.4	31.3	0.0031
Chewing Tobaccos					
1CH					
Sample 1		20	5.9	14.8	0.0015
Sample 2		20	5.5	13.8	0.0014
2CH					
Sample 1		20	6.0	15.0	0.0015
Sample 2		20	6.3	15.8	0.0016
3CH					
Sample 1		20	8.7	21.8	0.0022
Sample 2		20	9.1	22.8	0.0023
4CH**					
Sample 1		20	10.0	25.0	0.0025
Sample 2		20	9.9	24.8	0.0025
5CH**					
Sample 1		20	9.0	22.5	0.0023
Sample 2		20	8.8	22.0	0.0022
Snuff					
1S					
Sample 1		20	10.4	26.0	0.0026
Sample 2		20	11.7	29.3	0.0029
2S					
Sample 1		20	12.9	32.3	0.0032
Sample 2		20	11.4	28.5	0.0029
3S					
Sample 1		20	10.0	25.0	0.0025
Sample 2		20	10.2	25.5	0.0026

* In every case reported, 4.00 g. of tobacco product was extracted; total volume of each extract was 200 ml.

** The 4.00 g. are weights after drying at 50°C for 48 hours. Brand 4CH lost 0.88% of its weight, and 5CH lost 8.23% of its weight prior to grinding.

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Table 2.—Determination of scopoletin in cigar smoke

Cigar type and brand	Weight (g.) of cigar	Volume \pm 1 ml.	No. of puffs per cigar	Butt Length (mm.)	Scopoletin (μ g) per 20 ml. aliquot	Scopoletin (μ g) per 1 g. cigar
1C, cigarillo						
*Sample 1	11.90	54	35	30	8.0	3.4
*Sample 2	12.95	54	35	30	8.2	3.2
2C, perfecto						
**Sample 1	16.62	56	50	50	8.0	2.4
**Sample 2	16.69	56	50	50	4.5	1.4

For each sample: room temperature was 32°C; humidity, 24%; total volume of extract was 100 ml.; and 20 ml. aliquots were streaked on paper for analysis.

*Each sample consisted of 4 cigarillos.

**Each sample consisted of 2 cigars.

the temperature of the system reached that of the room. The solvent already in the three traps plus all the acetone-absolute alcohol washings were combined and transferred quantitatively into a 100 ml. volumetric flask and made to volume. Twenty milliliter aliquots of this solution of smoke condensate were used for the scopoletin analyses.

Qualitative analyses—For identification purposes, the scopoletin was purified by extended paper chromatography, using the basic procedure previously described (Yang, *loc. cit.*) The resulting scopoletin was checked with an authentic sample synthesized by the procedure of Aghoramurthy and Seshadri (1952). The infrared spectrum of scopoletin is shown in figure 1. The absorption spectra showed a prominent absorption maximum at 344 m μ (Yang *loc. cit.*) for the synthetic and purified samples containing sufficient scopoletin. Each scopoletin fraction from a tobacco product was also subjected to one- and two-dimensional mixed paper chromatography with authentic samples. Solvent systems used for paper chromatography were 15 percent acetic acid-water; 60 percent acetic acid-water; n-butyl alcohol-acetic acid water (6:1:2 v/v); n-butyl alcohol-benzene-pyridine-water (5:1:3:3 v/v); and nitromethane-benzene-water (2:3:5 v/v). Typical R_f values for scopoletin in these solvent systems, respectively, using the S & S No. 589 paper and a temperature of 28° \pm 3° were 0.47; 0.47; 0.82; 0.82 and 0.69.

Quantitative analyses—The quantitative determinations of scopoletin in the various tobacco products and in cigar smoke were performed by the analytical procedures already described by Yang *et al.* for scopoletin in cigarette tobacco and smoke, resp. (Yang, in press). These involved ex-

tended paper chromatography for the quantitative separation and purification of the scopoletin, elution with 50 percent ethyl alcohol-water, and spectrophotometric measurement at 344 m μ . The quantity of scopoletin present was calculated from an experimentally determined standard curve prepared from known amounts of pure, synthetic scopoletin carried through the same procedure as the tobacco or smoke scopoletin.

Results and Discussion

Data for the amount of scopoletin found to be present in each tobacco product containing at least 5 μ g of scopoletin per aliquot are reported in table 1. Below this concentration, the reproducibility and accuracy of the procedure become lower, and hence, for such samples only the statement is made that they contain less than 5 μ g per aliquot. Each value of column 3, table 1, is given in micrograms per aliquot and represents the average of values obtained on three aliquots. In column 4 are recorded the micrograms of scopoletin per gram of tobacco product analyzed. These values have been expressed as percentages in column 5.

All 31 brands of cigars analyzed qualitatively were found to contain some scopoletin, though apparently in different amounts. For preliminary classification purposes as to scopoletin content, the relative size of the purified scopoletin zone and the intensity of its fluorescence were used as guides, in a manner similar to that employed for the estimation of rutin in tobacco samples by Penn and Weybrew (1958). The brands were thus placed in one of the following groups: (a) relatively higher scopoletin content, nine brands; (b) medium, eight brands; (c) low scopoletin content, 12 brands; and (d)

trace amount of scopoletin, two brands. Two cigars each of six brands in group "a" which were considered likely to be highest in scopoletin content as estimated by the above method, were selected for quantitative analysis by the procedure of Yang *et al.* (in press). The quantitative results for four of these samples are recorded in table 1. The other two brands had values of less than 5 μ g per aliquot.

Analyses of the scopoletin content of five brands of regular cigarettes (Camel, Lucky Strike, Philip Morris, Chesterfield, and Old Gold) for the percentage of scopoletin in cigarette tobacco gave values in the range of 0.0066-0.012% (Yang, in press). Only 1C and 2C of the cigars tested fell in this range. These two were thus selected for quantitative determination of cigar smoke under the smoking conditions described. Cigars 1C and 2C actually carry the same brand name, but 1C is the cigarillo and 2C is the perfecto of that brand.

The smoke from 42 other cigars, including 19 other brands, were studied qualitatively for their scopoletin content. In cigar smoke there are impurities which seem to interfere even more than usual with the determination of scopoletin. And, because of the apparently very low scopoletin content of the smoke from these other cigars, quantitative studies on the smoke from cigars have been limited to 1C and 2C. The results of these analyses are shown in table 2. In the case of 2C, definite variations were found in individual cigars analyzed.

The values in table 2 indicate that 1C and 2C, by far the highest of all the cigars tested in their scopoletin content, produce a relatively lower scopoletin content in the smoke than was found in smoke from a comparable amount of tobacco in manufac-

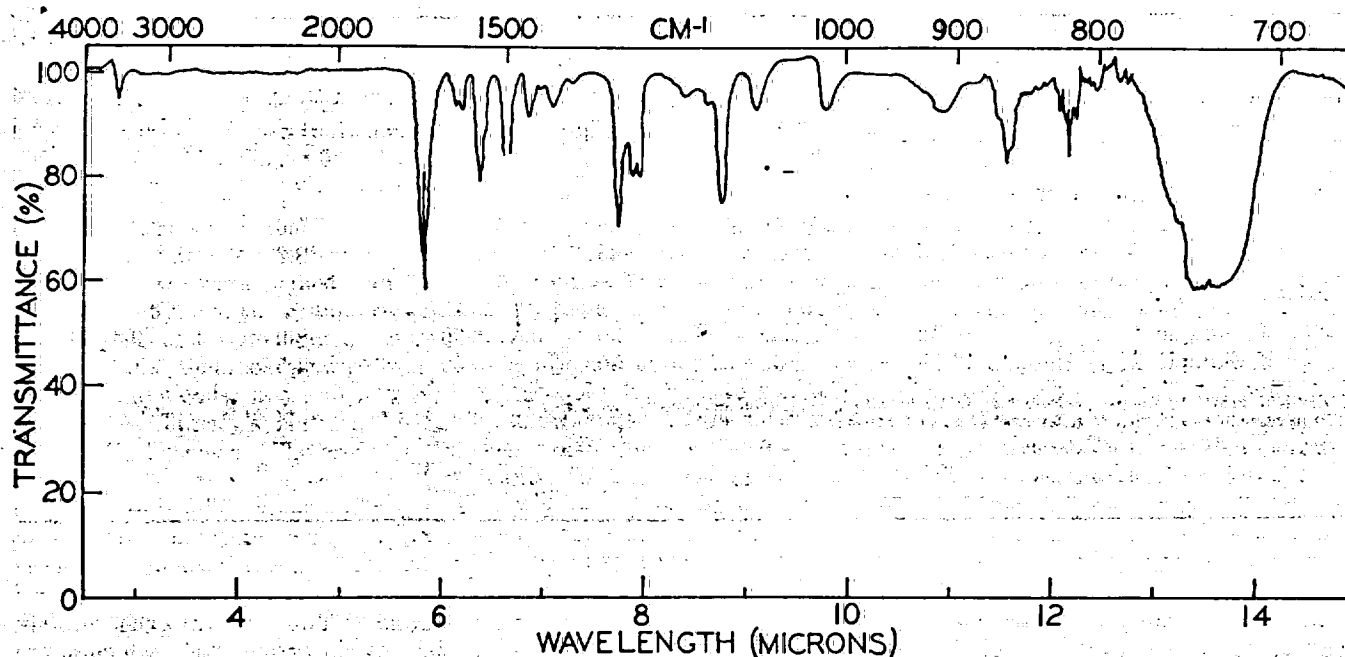


Figure 1. Infrared spectrum of scopoletin (from Perkin-Elmer Infracord).

tured cigarettes. In the latter case, the range was found to be from 10.3-27.8 μg scopoletin per 1 g. of cigarette.

From the values shown in table 1, it is evident that both brands of hand-rolled cigarette tobacco tested contained scopoletin in an amount (column 5) that falls in the range of 0.0066-0.012% which was found for regular, manufactured cigarettes. This was also the case for the 3 brands (1PM, 2PM, and 3PM) labeled as pipe mixtures. None of these, however, approached the upper limits found for tobacco in regular cigarettes.

The scopoletin content of all the brands labeled as pipe tobacco or pipe and cigarette tobacco was slightly lower than that found in a comparable weight of the tobacco

from cigarettes.

The amount of scopoletin in the snuff and chewing tobacco samples analyzed was even lower than that of the pipe tobaccos studied. Two brands, however, (4S and 6CH) were considerably lower in scopoletin content than the other snuff and chewing tobacco samples analyzed. Both had less than 5 μg of scopoletin per aliquot.

Summary

Scopoletin has been found to be present in all cigars, snuff, chewing tobaccos, pipe tobaccos, pipe mixtures, and roll-your-own cigarette tobaccos analyzed.

Quantitative results have been reported on selected samples of each type of tobacco product as well as on the smoke from selected cigars.

Literature Cited

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- Penn, P. T. and Weybrew, J. A., "Some factors affecting the content of the principal polyphenols in tobacco leaves." *Tobacco Science* 2: 20-24 (1958).
- Yang, Chao-Hwa, Nakagawa, Y. and Wender, S. H., "Identification of scopoletin in cigarette tobacco and smoke." *J. Org. Chem.* 23: 204-205 (1958).
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Identification of Esculetin in Tobacco and in Cigarette Smoke

L. J. DIETERMAN, C. H. YANG, Y. NAKAGAWA, AND S. H. WENDER

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In the purification of scopoletin (6-methoxy, 7-hydroxycoumarin) from cigarette smoke and from various tobacco extracts,^{1,2} two or more interfering blue fluorescing compounds persisted with the scopoletin through several paper chromatographic developments. The present paper reports our identification of one of these interfering compounds as esculetin (6,7-dihydroxycoumarin).

We have identified esculetin for the first time in the leaves and flowers of oven-dried, greenhouse-grown, One-Sucker tobacco, in the tobacco of representative U. S. cigarettes, and in flue-cured and air-cured tobacco leaf samples. The mainstream smoke from representative U. S. cigarettes was found to contain esculetin in a trace amount.

The esculetin is extremely difficult to separate completely from scopoletin with such solvent systems as 15% acetic acid-water, 60% acetic acid-water; *n*-butyl alcohol-acetic acid-water (6:1:2 v./v.), and *n*-butyl alcohol-benzene-pyridine-water (5:1:3:3 v./v.), but separation on paper chromatograms may be accomplished with the solvent system nitromethane-benzene-water (2:3:5 v./v.).

In addition to its persistence with scopoletin on many paper chromatograms of various tobacco samples, esculetin may be confused on some of these chromatograms with caffeic acid. The R_f values of esculetin and caffeic acid are quite close in a number of solvent systems (Table I), and there is similarity in the bluish white fluorescence of esculetin and of caffeic acid when either is present only in low concentration on the chromatogram. Esculetin, however, behaves differently than does caffeic acid on paper chromatograms still wet with the solvent system *n*-butyl alcohol-benzene-pyridine-water. Under these conditions, esculetin fluoresces a bluish yellow when examined under long wave-length ultraviolet light (3660 Å) whereas the same concentration of caffeic acid exhibits only an extremely weak—practically imperceptible—fluorescence under the same conditions.

EXPERIMENTAL

Esculetin from tobacco flowers. Oven-dried flowers from One-Sucker tobacco plants, *Nicotiana tabacum*, grown in the greenhouse at Argonne National Laboratory, Lemont, Ill., appeared to be richer in esculetin than the leaves and other tobacco samples examined, and were, therefore, used for

(1) C. H. Yang, Y. Nakagawa, and S. H. Wender, *J. Org. Chem.*, 23, 204 (1958).

(2) C. H. Yang, Y. Nakagawa, and S. H. Wender, *Anal. Chem.*, 30, 2041 (1958).

many of the early identification studies on esculetin. In a typical experiment, 100 g. of tobacco flowers were extracted in a Soxhlet extractor with two 500-ml. portions of 85% isopropyl alcohol. The combined extracts were concentrated to about 100 ml. *in vacuo*, filtered, and the filtrate acidified to pH 2. The filtrate was then subjected to silicic acid column chromatography by a procedure adapted from that of Sondheimer³ and based on a method described by Bulen *et al.*⁴ Fifty-three grams of silicic acid (Mallinckrodt No. 2847) were mixed with the filtrate to produce a thick paste mixture. This was made into a slurry by addition of 300 ml. of 5% *n*-butyl alcohol-chloroform saturated with 0.5*N* sulfuric acid. The slurry was poured onto silicic acid in a chromatographic column which had been prepared by thorough mixing of 160 g. of silicic acid with 110 ml. of 0.5*N* sulfuric acid, and then adding 1 l. of 5% *n*-butyl alcohol-chloroform saturated with 0.5*N* sulfuric acid. After addition of the slurry to the silicic acid in the column, the 5% *n*-butyl alcohol-chloroform system was used for packing the column. Then for developing and eluting the components of the tobacco flowers, 5%, 15%, 25%, 35%, and 50% *n*-butyl alcohol-chloroform systems saturated with 0.5*N* sulfuric acid were used. Eluate fractions of 500 ml. were collected, concentrated to 100 ml. *in vacuo*, and studied by paper chromatography. The second 500-ml. fraction eluted from the column with the 5% *n*-butyl alcohol system contained the major portion of the esculetin present in the tobacco flowers. This second fraction, after concentration *in vacuo*, was subjected to mass paper chromatography for purification of the esculetin. This eluate was streaked onto four sheets of S & S No. 589, red ribbon, chromatography paper, size 58 × 58 cm., and developed in ethyl acetate-formic acid-water (10:2:3 v./v., upper layer). The bluish white fluorescing zone (approximate R_f = 0.76), containing the esculetin and impurities, was cut out and sewn onto new sheets of the S & S chromatography paper. These were developed in 15% acetic acid-water until the esculetin moved across the bottom thread line. The sheets were then removed from the chamber, dried, and developed in the nitromethane-benzene-water system. The esculetin zone on each paper was cut out, sewn onto still other sheets of the chromatography paper, and developed again in the ethyl acetate-formic acid-water system. The bluish white fluorescing zone, R_f = 0.76, was cut out from each sheet and eluted with 95% ethyl alcohol. The combined alcohol elutions were then studied for proof of identity of esculetin.

The eluted esculetin separated from the tobacco flowers extract was co-chromatographed with the authentic reference esculetin in all the solvent systems mentioned in this

TABLE I

R_f VALUES OF ESCULETIN, SCOPOLETIN, AND CAFFEIC ACID

Compound	Solvent Systems ^a					
	(1)	(2)	(3)	(4)	(5)	(6)
Esculetin	0.84	0.84	0.78	0.83	0.09	0.47
Caffeic acid	0.81	0.85	0.80	0.84	0.03	0.42 ^b
Scopoletin	0.84	0.82	0.83	0.89	0.80	0.50

^a Solvent systems: (1) *n*-butyl alcohol-benzene-pyridine-water (5:1:3:3 v./v., upper layer); (2) isopropyl alcohol-pyridine-acetic acid-water (8:8:1:2 v./v.); (3) *n*-butyl alcohol-acetic acid-water (6:1:2 v./v.); (4) ethyl acetate-95% formic acid-water (10:2:3 v./v.); (5) nitromethane-benzene-water (2:3:5 v./v., upper layer); (6) 15% acetic acid-water. ^b A minor spot (R_f = 0.50) was also present.

(3) E. Sondheimer, *Arch. Biochem. & Biophys.*, 74, 131 (1958).

(4) W. A. Bulen, J. W. Varner, and R. C. Burrell, *Anal. Chem.*, 24, 187 (1952).

(5) K. Hermann, *Pharm. Zentralhalle*, 95, 56 (1956).

paper, both on one- and two-dimensional chromatograms. Typical R_f values are reported in Table I. The eluted esculetin also exhibited the same colors and fluorescence as produced by the authentic reference esculetin when treated with chromogenic sprays (Table II). The phosphotungstic acid and the 10% ammonium hydroxide spray reagents were used in detecting 1-, 2-, and 4-hydroxyphenols by Hermann.⁶

TABLE II
COLOR REACTIONS OF ESCULETIN, CAFFEIC ACID, AND SCOPOLETIN

Spray Reagent	Compound	Esculetin	Caffeic acid	Scopoletin
None, U.V.	bt-w	bt-BI	bt-d-BI	
NH ₃ vapor, U.V.	bt-Y-BI	bt-BI-(e)	bt-BI	
10% NH ₄ OH and NH ₃ vapor, U.V.	bt-BI-G	bt-BI	bt-d-BI	
1% alc. AlCl ₃ , U.V.	bt-lt-BI	bt-BI	bt-d-BI	
1% alc. AlCl ₃ , NH ₃ , U.V.	BI-Y	BI-W-Y	bt-d-BI	
Phosphotungstic acid and alc. KOH	U.V. bt-Y V. ft-Y	bt-Y ft-Y	bt-BI N	
0.5% KMnO ₄ , aq. V.	N	bt-Y → Br	N	
Diazotized p-nitroaniline and Na ₂ CO ₃	N	Y → Br	N	

* V = visible light, U.V. = ultraviolet light, BI = blue, Br = brown, G = green, W = white, Y = yellow, bt = bright, d = deep, (e) = enhanced, ft = faint, lt = light, N = no color, → = changing to.

The ultraviolet absorption spectrum of the reference esculetin in 50% ethyl alcohol-water, as determined on a Beckman spectrophotometer, Model DU, exhibited major maxima at 228 and 328 $m\mu$, and a major minimum at 274 $m\mu$. Minor maxima occurred at 254 and 299 $m\mu$, and minor minima at 222, 249, and 310 $m\mu$. The eluted esculetin separated from tobacco flowers gave an identical spectrum, using as a blank a 50% ethyl alcohol-water eluate from the S & S filter paper. The absorption curves of the separated esculetin and the reference esculetin check with the literature.⁶

Preparation of the reference esculetin. Esculetin was prepared from its commercially available glycoside esculin (esculetin-6-glucoside). Ten g. of esculin (Nutritional Biochemical Corp., Cleveland, Ohio) were suspended in 350 ml. of 7% sulfuric acid and heated on a steam bath for 6 hr. The hydrolysate was filtered hot. The fine, needle-like crystals were washed with water and then with ether. The filtrate was cooled overnight. Additional crystals separated and were washed as above. The precipitates were combined, and then the crude esculetin, after decolorization with charcoal, was recrystallized from 95% ethyl alcohol. All commercial samples of esculin that were examined by paper chromatography were found to contain blue fluorescent impurities, and even after hydrolysis and crystallization, the resulting esculetin (4.1 g.) was still not chromatographically pure. Therefore, purification was undertaken by column chromatography of the crystalline esculetin. Two g. were dissolved in methyl alcohol and applied to a column packed with pre-washed Magnesol (Food Machinery and Chemical Corp., N. Y.). The column was developed with ethyl acetate saturated with water. The first 250 ml. of eluate contained impurities and were not used. The subsequent eluates were combined and concentrated *in vacuo*.

The resulting precipitate was recrystallized from 95% ethyl alcohol and then sublimed *in vacuo*. This chromatographically pure esculetin (300 mg.) was used as the reference esculetin, m.p. 270°, with decomposition.⁷

Esculetin in tobacco leaves and in cigarette tobacco. For identification of the relatively smaller amount of esculetin present in tobacco leaves, the procedure described above for tobacco flowers was used. In addition, for some samples, a paper chromatographic procedure was employed which did not involve the preliminary silicic acid chromatography. The first steps of this procedure were the same through the development with the nitromethane-benzene-water system as those already described by Yang *et al.*³ for the quantitative determination of scopoletin in cigarette tobacco. With the nitromethane system, the scopoletin ($R_f = 0.84$) moved far ahead of the esculetin ($R_f = 0.07$). This time, the esculetin zone, still containing another interfering blue fluorescent compound, was cut out and eluted with methyl alcohol. The eluates were streaked on new sheets of S & S paper and developed in 15% acetic acid-water, and then again in the nitromethane system. Each section containing the esculetin was cut out, sewn on a new sheet, developed in the ethyl acetate-formic acid-water system to move the esculetin across the sewing line, and the paper removed and dried. The unfinished chromatogram was then developed again in 15% acetic acid-water to effect the separation of esculetin from the other blue fluorescent compound. Usually esculetin moved sufficiently ahead of the interfering substance at this point to be eluted with methyl alcohol as a chromatographically pure compound and then be identified beyond doubt as esculetin. If not completely separated, the esculetin zone was placed on yet another paper and rechromatographed in the 15% acetic acid-water before making further identification studies.

By one or both of the above procedures, esculetin was identified as being present in a small amount in leaves of Burley tobacco (Kentucky 16), Turkish tobacco (imported and domestic), and flue-cured tobacco (Hicks) from North Carolina.

Because of the low amount of esculetin present relative to that of scopoletin in cigarette tobacco, 8 g. samples were used for these analyses instead of the 2 g. samples used for analysis of scopoletin. Also, Whatman No. 3 MM chromatography paper was used for the first step only in the paper chromatography. The S & S No. 589 red ribbon paper was used for the other paper chromatographic steps. Cigarettes analyzed included Camel, Lucky Strike, Philip Morris, Old Gold Straights, Pall Mall, Viceroy, Winston, and Oasis.

Esculetin in the mainstream smoke from cigarettes. The sampling and smoking of 8 brands of cigarettes for esculetin analysis were similar to those already described for scopoletin by Yang *et al.*³ The separation, purification, and identification of esculetin from the cigarette smoke condensates were carried out by mass paper chromatography in the same manner described above for esculetin in tobacco leaves. Because esculetin was present only in trace amounts in the smoke, eluates representing smoke from 4 packs of cigarettes had to be combined and concentrated to obtain sufficient esculetin for unambiguous chromatographic studies.

Acknowledgment. This work and some previous research on which these findings are based were supported in part by the Tobacco Industry Research Committee and by the Atomic Energy Commission.

We also sincerely thank Dr. Norbert Scully and Mr. Will Chorney of the Argonne National Laboratory, Dr. C. W. Nystrom, R. J. Reynolds Tobacco

(6) R. H. Goodwin and B. M. Pollock, *Arch. Biochem. & Biophys.*, 49, 1 (1954).

(7) Handbook of Chemistry and Physics, 38th ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1956.

Co., Dr. J. M. Moseley, American Tobacco Co.,
and Luther Shaw, Waynesville, N. C., for supplying
various tobacco fractions used.

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UNIVERSITY OF OKLAHOMA
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Report #6

CONFIDENTIAL SEMI-ANNUAL REPORT

July, 1959

to

TOBACCO INDUSTRY RESEARCH COMMITTEE

**A QUALITATIVE AND QUANTITATIVE STUDY OF THE
INDIVIDUAL POLYPHENOL CONTENT OF CIGARETTE TOBACCO**

UNIVERSITY OF OKLAHOMA RESEARCH INSTITUTE

Norman, Oklahoma

Principal Investigator:

Date: July 8, 1959

**Dr. Simon H. Wender
Research Professor**

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A QUALITATIVE AND QUANTITATIVE STUDY OF THE
INDIVIDUAL POLYPHENOL CONTENT OF CIGARETTE TOBACCO

Two blue fluorescing compounds which interfered with the complete purification and quantitative determination of scopoletin in cigarette tobacco and smoke have been studied intensively at Oklahoma during recent months. One of these was identified as esculetin (6,7-dihydroxycoumarin), as was first revealed in our January report. Details of this finding were submitted in a manuscript to the Journal of Organic Chemistry for proposed publication. This report has been accepted for publication in that journal and will likely appear in press within a month. Reprints will be sent to you as soon as they become available.

The other blue fluorescing interfering compound in cigarette tobacco and smoke has now been identified as caffeic acid (3,4-dihydroxycinnamic acid). Although several groups of workers have reported finding free caffeic acid in various cured tobaccos, no previous report has been made of its presence in cigarette smoke. In our studies, we have learned that during 15% acetic acid-water chromatography on paper, caffeic acid appears as two distinct zones. These have been shown to be the cis and trans forms of caffeic acid. We have also identified which isomer on paper is trans, and which one is cis. The details of these new findings have been written up for proposed publication as a

Note in the Journal of Organic Chemistry. A copy of this manuscript is attached.

Two other compounds which do not fluoresce under ultraviolet light have been located on paper chromatograms of cigarette smoke after applications of chromogenic spray reagents. These are the two compounds mentioned in the January, 1959 report as possibly being acids possessing several hydroxy groups. We are now trying to obtain sufficient amounts of each in completely pure form for unequivocal identification.

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Identification of Caffeic Acid in Cigarette Smoke

Chao-Hwa Yang, Y. Nakagawa, and S. H. Wender

Received July 1959

No previous report has been made of the presence of caffeic acid (3, 4-dihydroxycinnamic acid) in cigarette smoke. Several groups of workers^{1,2,3},

(1) F. Wilkinson, M. Phillips, and A. M. Bacot, J. Assn. Off. Agric. Chemists, 37, 1004 (1954).

(2) M. Shiroya, T. Shiroya, and S. Hattori, Physiol. Plant, 8, 594 (1955).

(3) M. K. Mikhailov, Compt. Rend. Acad. Bulgare des Sci., 11, 205 (1958).

however, have reported finding free caffeic acid in various cured tobaccos, but Roberts and Wood,⁴ using fresh cigar tobacco, and Weaving,⁵ using flue-cured

(4) E. A. H. Roberts and D. J. Wood, Arch. Biochem., 33, 299 (1951).

(5) A. S. Weaving, Tob. Sci., 2, 1 (1958).

tobaccos, could find none in their samples. Dieterman et al.,⁶ have recently

(6) L. J. Dieterman, C. H. Yang, Y. Nakagawa, and S. H. Wender, J. Org. Chem., 24, — (1959).

pointed out that esculetin (6, 7-dihydroxycoumarin) in tobacco may often be confused on paper chromatograms with caffeic acid. In the present study on tobacco in 8 brands of cigarettes commonly smoked in the U.S., every sample tested was found to contain free caffeic acid. Also, in every case, the mainstream smoke from the cigarette contained free caffeic acid.

In the purification of scopoletin (6-methoxy, 7-hydroxycoumarin) from cigarette smoke and from various tobacco extracts^{7,8}, two or more interfering

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- (7) C. H. Yang, Y. Nakagawa, and S. H. Wender, J. Org. Chem., **23**, 204 (1958).
(8) C. H. Yang, Y. Nakagawa, and S. H. Wender, Anal. Chem., **30**, 2041 (1958).
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blue fluorescing compounds persisted with the scopoletin through several developments of paper chromatograms. Dieterman et al.⁶ identified one of these interfering compounds as esculetin. The present identification establishes free caffeic acid as the other blue fluorescing compound.

During paper chromatography in certain acid solvent systems, such as 15% acetic acid-water, caffeic acid appears as two distinct zones. These have been shown to be cis- and trans- caffeic acid.

EXPERIMENTAL

Caffeic acid from cigarette tobacco. The tobacco obtained from 120 cigarettes (3 each from 40 packs of the same brand) was mixed and ground to a powder. Six 5.5 g. samples of this powder were thoroughly extracted with 85% isopropyl alcohol, as previously described by Yang et al.⁷ The combined extracts were concentrated under reduced pressure, and the concentrate was then subjected to separation by mass paper chromatography.⁷ After the initial chromatography with Whatman 3MM paper, using the solvent system n-butyl alcohol-acetic acid-water (6:1:2 v/v), each zone containing caffeic acid, still mixed with some esculetin and scopoletin, was cut off and then eluted with methanol. The eluates were combined and streaked on S & S No. 589, Red Ribbon, chromatography paper, and developed in the system chloroform-acetic acid-water (2:1:1 v/v, bottom layer). This solvent system proved to be superior to the nitromethane-benzene-water system (2:3:5 v/v, upper layer) used in our previous studies on scopoletin and esculetin. In the chloroform system, the scopoletin ($R_f=0.75$) moves in a narrow zone quite removed from

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those of esculetin ($R_f=0.39$) and of caffeic acid ($R_f=0.35$). This was also the case with the benzene-propionic acid-water system (2:2:1 v/v, top layer) with R_f values: scopoletin (0.66); caffeic acid (0.32); and esculetin (0.26). The two top zones resulting from paper chromatography with the chloroform system contained primarily caffeic acid and esculetin. They were cut off from each chromatogram together; sewn onto a new sheet of paper; and then developed in ethyl acetate-formic acid-water (10:2:3 v/v). Each zone containing caffeic acid, with a trace of esculetin still present, was cut off and eluted with the ethyl acetate solvent system. The eluates were combined and again streaked on S & S No. 589 paper and developed in 15% acetic acid-water. Although separation of caffeic acid from esculetin was completed by this chromatography with acetic acid, an isomer of caffeic acid now appeared as a separate, third zone.

The two zones of isomeric caffeic acid were cut from each chromatogram as a unit and sewn onto a new sheet of chromatography paper. Each such sheet was then developed in the ethyl acetate system to obtain one narrow blue zone for identification studies.

Identification of caffeic acid. The combined eluates containing the purified caffeic acid from each single zone obtained in the ethyl acetate system were then co-chromatographed with authentic caffeic acid purchased from California Foundation for Biochemical Research, using the n-butyl alcohol-acetic acid-water, chloroform-acetic acid-water, ethyl acetate-formic acid-water, benzene-propionic acid-water, and nitromethane-benzene-water systems already described, and n-butyl alcohol-benzene-pyridine-water (5:1:3:3 v/v, upper layer), isopropyl alcohol-pyridine-acetic acid-water (8:8:1:2 v/v), and 15% acetic acid-water. Both the reference and isolated caffeic acid solutions gave the same R_f values in every test. In the 15% acetic acid system, both the reference and unknown caffeic acid samples gave two zones each, with corresponding R_f values.

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The isolated and reference caffeic acids behaved similarly towards the chromogenic agents previously reported.⁶ In addition, both gave the same color reaction with the Höfner reagent⁴ (pink, changing to yellowish-brown) and with 2% alcoholic ferric chloride solution (green, changing to gray).

The absorption spectra exhibited by the isolated caffeic acid in ethanol, and in buffer solutions at pH 3.5 and 6.8, checked in each case with the corresponding spectrum exhibited by the reference caffeic acid in ethanol and in buffer solutions at 3.5 and 6.8 in our laboratory and with those reported for these preparations by Sutherland.⁹

(9) G. K. Sutherland, Arch. Biochem. & Biophys., **75**, 412 (1958).

Caffeic acid in the mainstream smoke from cigarettes. The smoking of 8 brands of cigarettes for caffeic acid analysis was performed by a procedure similar to the one already described for scopoletin in smoke by Yang *et al.*⁸ Because caffeic acid, however, was present only in a trace amount in the smoke, samples representing smoke from 40 packs of cigarettes were combined and concentrated to obtain sufficient caffeic acid for unambiguous studies by paper chromatography. The separation, purification, and identification of caffeic acid from the cigarette smoke condensates were carried out by mass paper chromatography in the same manner as already described above for determination of caffeic acid in tobacco. Cigarettes analyzed included Camel, Lucky Strike, Philip Morris, Old Gold Straights, Pall Mall, Winston, Viceroy, and Oasis.

Isomerization of caffeic acid. On paper chromatography with 15% acetic acid-water, the reference caffeic acid gave two distinct zones. The farther moving zone ($R_f=0.50$) was called "CA-1", and the slower moving zone ($R_f=0.42$) was called "CA-2." Williams¹⁰ has reported that cinnamic acid derivatives give two spots on

(10) A. H. Williams, Chem. & Ind., 120 (1955).

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paper chromatography with 2% acetic acid-water. He suggested that this was a case of cis-trans isomerization on paper. He did not, however, find out which spot corresponded to which isomer.

In our studies, we have streaked ethanol aliquots containing 100 μ g. of the reference caffeic acid onto S & S No. 589 paper and developed these chromatograms in 15% acetic acid-water. Each blue fluorescing zone was cut out separately; sewn onto separate new sheets of paper; and again developed in the 15% acetic acid. It was observed that from the slower moving zone (CA-2), the faster moving zone (CA-1) was produced every time that the separated CA-2 was rechromatographed in this acid system. If this procedure, involving separation by paper chromatography, cutting, sewing, and rechromatography of the CA-2 was repeated five times, the original CA-2 zone from the 100 μ g. sample of caffeic acid would change almost completely to CA-1, and the fluorescence of the CA-2 zone would practically disappear. On the other hand, the CA-1 zone produced only a relatively small amount of the CA-2 each time that the CA-1 was developed in the 15% acetic acid-water.

Both CA-1 and CA-2 co-chromatographed with the reference caffeic acid to give only one spot in all the solvent systems mentioned in this paper, except in the 15% acetic acid-water. In this latter system, the major spot from the reference caffeic acid on the first chromatograms was identical with CA-2, and the minor spot was the same as CA-1. Both CA-1 and CA-2 gave the same color reactions when tested with all the chromogenic agents described in this report.

Although the isomeric caffeic acids CA-1 and CA-2 could be readily obtained as completely separated zones on paper chromatograms, much difficulty was experienced in getting solutions of either isomer completely free of the other. During the preparation of such solutions by extraction or elution of the individual zones from the paper, and application of heat, isomerization was usually found to occur, and an equilibrium mixture was set up according to the temperature, solvent, etc.

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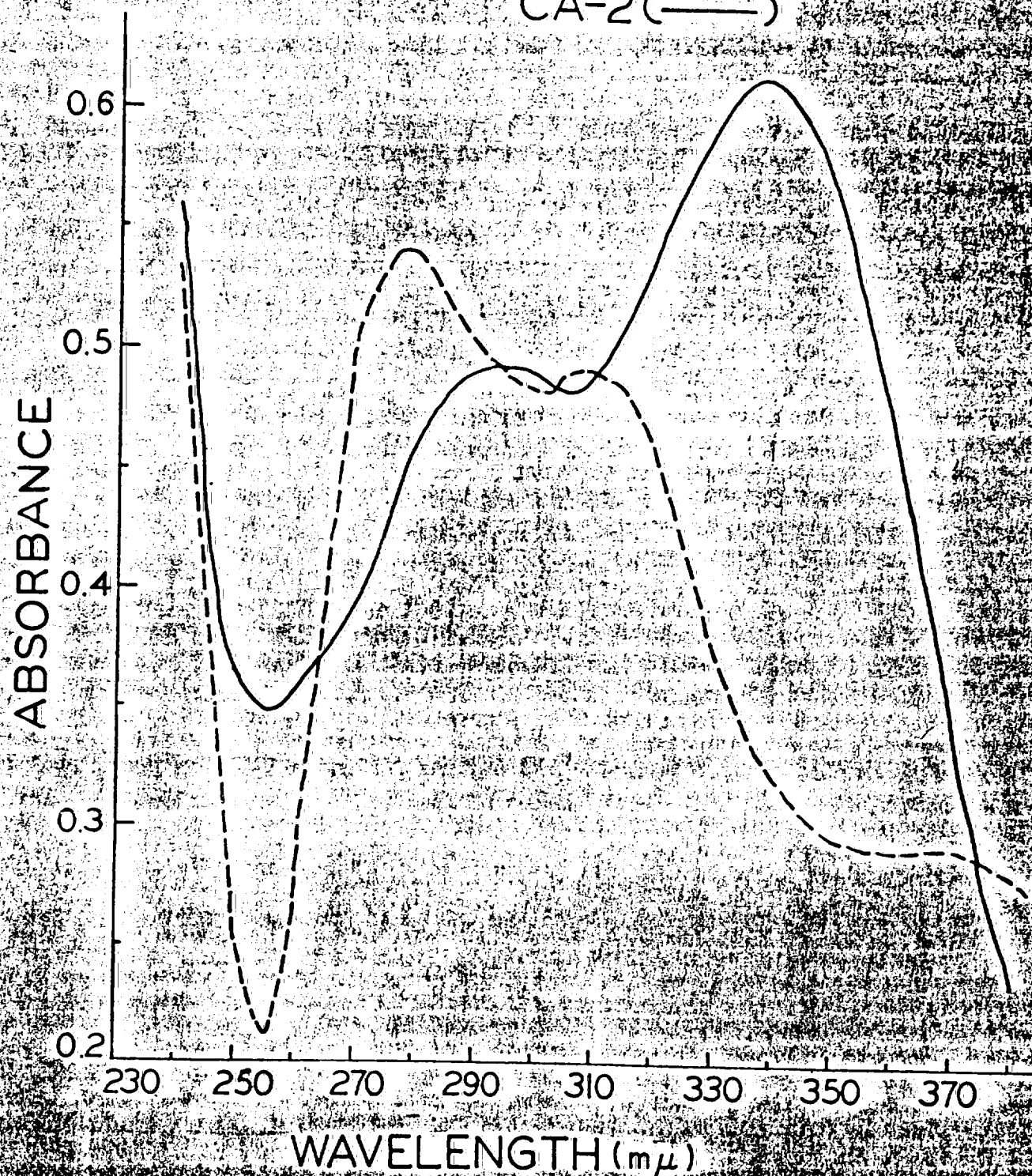
used. For the ultraviolet spectrophotometry, a solution consisting mainly of only isomer CA-1, and another preparation consisting mainly of only CA-2 were finally prepared, however, as described in the next paragraph.

Each isomeric zone was cut from the chromatogram separately and eluted with 95% ethanol in an elution chamber. Each eluate was then evaporated to dryness, in vacuo, without application of heat. The residue, containing the caffeic acid isomer plus a filter paper impurity, was then dissolved by 1 ml. of hot distilled water, added drop by drop, while the container was kept rotating. A blank solution containing the filter paper impurity, but no caffeic acid, was prepared in exactly the same manner as just described, except that no caffeic acid was present on the sheet of chromatography paper. The aqueous solution of each isomer was then added to cold distilled water in separate cuvettes, drop by drop, with a capillary tube. To the cuvette used as a blank, approximately an equal amount of the blank solution containing the filter paper impurity, but no caffeic acid, was added. The absorption spectra of these CA-1 and CA-2 solutions were then measured with the Beckman spectrophotometer, Model DU. Results are shown in Figure 1. The CA-1 preparation exhibited its high maximum at 278 m μ , whereas the CA-2 preparation had an even higher maximum at 340 m μ . Mixing of various amounts of CA-1 with CA-2 shifted the 340 m μ maximum of CA-2 to various corresponding lower wavelengths.

To interpret these results, one uses the working rule which states that when the absorption properties of the cis-trans isomers of a substance differ, "the more elongated isomer absorbs at somewhat longer wavelengths and more intensely".¹¹

(11) A. E. Gillam and E. S. Stern, An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry, Edward Arnold Publishers Ltd., London, England 2nd. ed., 1957, p. 267.

ABSORPTION SPECTRA OF AQUEOUS
SOLUTIONS OF CAFFEIC ACID PREPARED
FROM ZONES CA-1 (-----)
CA-2 (—)



1003537317

Haskins and Gorz¹² recently have found that such absorption data apply in their

(12) F. A. Haskins and H. J. Gorz, Arch. Biochem. & Biophys., 81, 204 (1959)

studies on cis- and trans - o-hydroxycinnamic acid. If this rule should also hold with caffeic acid, CA-1 would then appear to be the cis isomer and CA-2 the trans isomer of caffeic acid. Repeated paper chromatography in 15% acetic acid-water would, in such a case favor the isomerization of trans-caffeic acid to the cis form. The isopropyl alcohol extracts of cigarette tobacco and also the smoke condensates obtained in our research apparently already contain a mixture of the cis-trans isomers of caffeic acid even before paper chromatography with 15% acetic acid-water.

Acknowledgment. This work and some previous research on which these findings are based were supported in part by the Tobacco Industry Research Committee and by the Atomic Energy Commission.

CHEMISTRY DEPARTMENT
UNIVERSITY OF OKLAHOMA
NORMAN, OKLAHOMA

1003537318

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET
NEW YORK 17, N.Y.

#234
Cf. #155
(Activated 7/1/57
Renewed 7/1/58)

Application For Research Grant

Date: April 13, 1959

1. Name of Investigator: Duane G. Wenzel, Ph.D.
2. Title: Professor of Pharmacology
3. Institution
& Address: The School of Pharmacy
University of Kansas
Lawrence, Kansas
4. Project or Subject: A Study of the Role of Sympathomimetic Amines in the Interaction of Nicotine and Cholesterol upon the Myocardium.
5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Prior Work on This Project. Studies completed by the applicant demonstrate that nicotine administered to rabbits fed a low (0.1%) cholesterol diet produces significant increases in plasma cholesterol (1); that chickens give equivocal results (2); and that rabbits fed a 1% cholesterol-5% oil diet respond to nicotine with an increase in cardiac pathology (3). Present studies include an examination of peripheral vascular effects in rabbits and the response of the rat to nicotine and hypercholesterolemia.

Background to Proposal. Epinephrine and norepinephrine have been demonstrated to produce tissue hypoxia and necrosis (4). In large doses, injury to the media has been produced (5) while repeated smaller doses produces damage to the intima (6) and cause an increase of cholesterol in this tissue (7). Similar effects have been reported from the stimulation of the sympathetic ganglia (8). Recent evidence indicates that epinephrine and norepinephrine are avidly stored in the myocardium (9) and that they can be released by an electrical stimulus (10). Further, it has been demonstrated that the stimulant effect of nicotine upon the atria is dependent upon the release of epinephrine (11). Russian studies (12) have found that amphetamine produces an increase in both blood lipids and atherosclerosis. Finally, as presented by Rosenman (13) there are good clinical indications that stress, at least in part through the release of epinephrine, produces: (1) organic vascular damage predisposing to lipid deposition; (2) an increase in plasma cholesterol; and (3) a decrease in blood coagulation time.

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Proposal. On the basis of the foregoing and similar additional evidence, it is suggested that the observed interaction between nicotine and hypercholesterolemia upon the myocardium is related to the release of catecholamines. It is proposed that this hypothesis be tested directly in the following manner: Separate groups of rabbits would be established with the following regimens: (1) Control, (2) Nicotine, (3) Cholesterol 1% and oil 5%, (4) Nicotine and cholesterol, (5) A sympathomimetic amine such as ephedrine and cholesterol, (6) Nicotine and cholesterol and 1-(3',4'-dichlorophenyl)-2-isopropylaminoethanol HCl (DCI Lilly), (7) Nicotine and cholesterol and reserpine, and (8) Nicotine and cholesterol and iproniazid.

The rationale for the use of the proposed dietary additives is as follows: The use of a stable sympathomimetic amine such as ephedrine in the drinking water would provide a sympathomimetic effect similar to that of epinephrine or norepinephrine, yet could yield information as to their specificity. The administration of DCI, a potent blocking agent to the effect of the epinephrine on the heart, would indirectly determine the effect of the endogenous catecholamines. Reserpine, having been demonstrated to deplete the myocardium of catecholamines may then also provide protection to any effects of nicotine mediated through the catecholamines. Iproniazid, an agent with beneficial action in angina pectoris, produces a long lasting rise in the catecholamine content of the heart, yet its pharmacological effects on this organ cannot be explained on the basis of a catecholamine effect. It has been suggested that it may inhibit the release of these substances or prevent the formation of harmful metabolic products (14).

The study would continue for 24 weeks, during which time blood coagulation times and plasma cholesterol levels would be determined. At the termination of the study, cardiovascular micropathology would be performed.

REFERENCES

- (1) Wenzel, D. G. and Beckloff, G.L., J. Am. Pharm. Assoc., 47, 338 (1958).
- (2) Wenzel, D. G., Turner, J.A., and Kissil, D., J. Am. Pharm. Assoc. 48, 116 (1959).
- (3) Wenzel, D. G., Turner, J. A., and Kissil, D., Circ. Res., 7, 256(1959).
- (4) Raab, W., Hormonal and Neurogenic Cardiovascular Disorders. Williams and Williams Co., Baltimore, 1953.
- (5) Hueper, W. C., Arch. Path., 39, 51(1945)
- (6) Waters, L. L., The Reaction of the Artery Wall to Hypertension and to Hypervolemia Symposium on Atherosclerosis, National Academy of Science, National Research Council, Publication No. 338, 1955.
- (7) Raab, W., Ztschr. ges. exper. med., 105, 657 (1939).
- (8) Danisch, F., Beitr. path. anat., 79, 333 (1928).
- (9) Raab, W. and Gigie, W., Circulation Research, 3, 553 (1955).
- (10) Furchgatt, R. F., DeGubareff, T. and Grossman, A., Science, 129, 328 (1959)
- (11) Burn, J. H., and Rand, M. J., Brit. Med. J., 1958I, 137(1958).
- (12) Myasnikov, A. L., Circulation, 17, 99 (1958).
- (13) Rosenman, R. H. and Friedman, M., in Hormones and Atherosclerosis, Academic Press, New York, 1959, pp. 283-295.
- (14) Pletscher, A., and Pellmont, B., J. Clin. and Exp. Psychopath. and Quart. Rev. Psychiatry and Newrology, Supplement to 19, 2, 163 (1958)

1003537320

6. Budget Plan:

Salaries	\$5,400.
Expendable Supplies	600.
Permanent Equipment	900.
Overhead (15%)	1,065.
Other (travel)	200.
Total	<u>\$8,165.</u>

7. Anticipated Duration of Work:

September 1, 1959 through August 31, 1960

8. Facilities and Staff Available:

An air-conditioned animal room and several completely equipped research laboratories are available. The project director will be available on a full-time basis for two summer months and part-time throughout the year. Other personnel will include Dr. James A. Turner, assistant Professor of Pathology, a one-half time research assistant, a pathology technician, and an animal room caretaker.

9. Additional Requirements:

None.

10. Additional Information (including relation of work to other projects and other sources of supply):

None.

NCE

/s./ Duane G. Wenzel
Director of Project
/s./ William J. Argersinger, Jr.
Assoc. Dean Graduate School
Business Officer of the Institution

1003537321

TOBACCO INDUSTRY RESEARCH COMMITTEE

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 E. 42nd Street New York 17, N.Y.

Application for Research Grant

#235

May 8, 1959

(Cf. #72
Activated 7/1/55
Renewed 7/1/56
#157
Activated 7/1/57
Renewed 7/1/58

1. Name of Investigator: R. H. Rigdon, M.D.
2. Title: Professor of Pathology
3. Institution & Address: The University of Texas Medical Branch
Galveston, Texas
4. Project or Subject: Study of the Effect of Tobacco Tar on the White Pekin Duck.
5. Detailed Plan of Procedure:

In the study of the effect of tobacco tar on the respiratory tract of white Pekin ducks we gave nine ducks tobacco tar intratracheally in mineral oil for 130 times and observed amyloid in the liver of three. One of these birds died on the 242nd day after the first intratracheal injection and two were sacrificed on the 756th day.

I think this experiment should be repeated. We used 1 milliliter of cigarette tar and 19 milliliters of liquid petrolatum. When larger amounts of tar are given, the ducks show clinical evidence of nicotine poisoning. It would be wise to give this tobacco tar orally to some ducks to see if amyloidosis results. I say this because we have produced amyloidosis in ducks given methylcholanthrene orally.

Ducks given the tobacco tar will be sacrificed at varying intervals and histologic studies of the viscera will be made.

6. Budget Plan:	Salaries	\$3105.00*
	Expendable Supplies	1200.00
	Permanent Equipment	
	Overhead (10%)	460.00
	Other	300.00
	Total	\$5065.00

* This includes \$82.50 O/A/S/I. and \$22.50 W.C.I.

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7. Anticipated Duration of Work:

Two years

8. Facilities and Staff Available:

The same facilities we have had during the past several years.

9. Additional Requirements:

None

10. Additional Information (Including relation of work to other projects and other sources of supply):

Amyloid was first observed in 1955 in ducks treated with methylcholanthrene (R.H.Rigdon, Atypical cirrhosis in the duck produced by methylcholanthrene. Am. J. Path. 31:451-473, 1955). We recently have observed amyloid in the liver, spleen, adrenals, kidneys and thyroid of ducks given one large intratracheal injection of methylcholanthrene. This study was reported at Duke University in March of 1959. The manuscript is now ready to submit for publication.

Additional information on amyloidosis referable to pathogenesis would be valuable. Since it can be produced in ducks with methylcholanthrene and there is evidence that it will follow intratracheal injections of tobacco tar, we should establish the latter as a scientific fact. At the present time we know that large amounts of methylcholanthrene, when put into the respiratory tract, will produce neoplasms. Tobacco tar has not produced any tumors, but amyloidosis has occurred. Additional studies on tobacco tar and the production of amyloidosis may contribute to the basic knowledge of the agents that are carcinogenic.

Articles either published or recently submitted and aided by a grant from the Tobacco Industry Research Committee:

1. Keratoacanthoma. Experimentally induced with methylcholanthrene in the chicken. Arch. Dermat. 79: 139-147, 1959.
2. Mechanism of removal of fluid and particulate material from the respiratory tract of the duck. Arch. Path. 67:215-227, 1959.
3. Cancer of the lung - the sex ratio. A review of the problem. Texas Reports Biol. and Med. 17:29-48, 1958.
4. The respiratory system in the normal white Pekin duck. Poultry Sci. 38: 196-210, 1959.
5. The effect of tobacco tar on the respiratory tract of white Pekin ducks. Arch. Path. Submitted for consideration March 3, 1959.
6. Effects of methylcholanthrene on the respiratory tract of the white Pekin duck. Arch. Path. Submitted for consideration March 3, 1959.

* * * * *

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TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET
NEW YORK 17, N.Y.

#234
Cf. #155
(Activated 7/1/57
Renewed 7/1/58)

Application For Research Grant

Date: April 13, 1959

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& Address: The School of Pharmacy
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- (4) Raab, W., Hormonal and Neurogenic Cardiovascular Disorders. Williams and Williams Co., Baltimore, 1953.
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- (9) Raab, W. and Gigie, W., Circulation Research, 3, 553 (1955).
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- (12) Myasnikov, A. L., Circulation, 17, 99 (1958).
- (13) Rosenman, R. H. and Friedman, M., in Hormones and Atherosclerosis, Academic Press, New York, 1959, pp. 283-295.
- (14) Pletscher, A., and Pellmont, B., J. Clin. and Exp. Psychopath. and Quart. Rev. Psychiatry and Newrology, Supplement to 19, 2, 163 (1958).

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6. Budget Plan:

Salaries	\$5,400.
Expendable Supplies	600.
Permanent Equipment	900.
Overhead (15%)	1,065.
Other (travel)	200.
Total	<u>\$8,165.</u>

7. Anticipated Duration of Work:

September 1, 1959 through August 31, 1960

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An air-conditioned animal room and several completely equipped research laboratories are available. The project director will be available on a full-time basis for two summer months and part-time throughout the year. Other personnel will include Dr. James A. Turner, assistant Professor of Pathology, a one-half time research assistant, a pathology technician, and an animal room caretaker. and also provide protection

9. Additional Requirements:

None.

10. Additional Information (including relation of work to other projects and other sources of supply):

None.

/s./ Duane G. Wenzel

Director of Project

/s./ William J. Argersinger, Jr.

Assoc. Dean Graduate School

Business Officer of the Institution

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NOTES

0910-0170, CHANGE OF ADDRESS

10. IDENTIFYING INFORMATION (EASTMAN: SECTION OF ACTING CHIEF, 3-10-64)

2004

1. WATER-PROOFING SOOTY COATINGS:

[illegible]

TO THE SECRETARY OF DEFENSE

RECEIVED
APR 29 1950
MORRIS,
WELCH

APR 29 1959
PHILIP MORRIS, INC.
RESEARCH & DEVELOPMENT
DEPT.

1003537327

TIRC Grant
#234

Reprinted from the
Journal of the American Pharmaceutical Association
Scientific Edition, Vol. XLVIII, No. 2, February 1959

The Effect of Nicotine on Hypercholesterolized Cockerels*

By DUANE G. WENZEL, JAMES A. TURNER, and DONALD KISSIL

Cockerels were hypercholesterolized with a 1 per cent cholesterol diet and administered nicotine (2.28 milligrams per kilogram per day) in the drinking water. After sixteen weeks of treatment, the plasma cholesterol and lipid phosphorus levels and the cardiovascular pathology were not significantly different from those of the cholesterol controls.

A NUMBER of recent reports have suggested a possible causal relationship between nicotine and atherosclerosis. Mortality studies reveal a twofold increase in the coronary death rate of smokers over nonsmokers (1). Positive correlation has also been established between the amount of smoking on one hand and both the earlier appearance of coronary occlusion and death from this cause (2). Laboratory studies utilizing rabbits have demonstrated that when nicotine is

added to a hypercholesterolemic stimulus, significantly higher plasma cholesterol and lipid phosphorus levels result. The effect is essentially the same whether the cholesterol and nicotine are injected (3) or administered perorally (4). In both of these studies utilizing rabbits a minimal amount of cholesterol corresponding to approximately 0.1 per cent of the diet was utilized.

The purpose of this investigation was to determine whether the chicken is susceptible to the nicotine potentiation of hypercholesterolemia and whether a pathological differential may be established by the use of a normally atherogenic dietary level (1.0 per cent) of cholesterol (5).

EXPERIMENTAL AND DISCUSSION

White English Leghorn cockerels were raised on a diet of Purina Layena® pellets from two days to ten weeks of age. At this time four experimental groups of fourteen birds per group were established. Birds were randomly selected from a group of approximately equal weights. The experimental groups

*Received July 24, 1958, from the School of Pharmacy, University of Kansas, and Veterans Administration Hospital, Kansas City, Mo.

This investigation was supported in part by research grant (H-3017) from the National Heart Institute, Public Health Service.

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were as follows: (a), control; (b), nicotine (2.28 mg./Kg. per day in the drinking water); (c), cholesterol (1.0% in the feed); and (d), combined nicotine-cholesterol regimens. The daily dose of nicotine was roughly equivalent to the use of two packs of cigarettes by the human (4).

At ten weeks of age the birds were weighed and plasma cholesterol and phospholipid levels determined by methods previously described (4). Determinations were repeated at four-week intervals over the sixteen-week experimental period. At twenty-six weeks of age all birds were sacrificed and the hearts and aortas studied for pathological changes.

The control group gained weight at a slightly but not significantly greater rate than the other birds. Figure 1 is a record of the plasma total cholesterol levels and the cholesterol/phospholipid ratios over the sixteen-week period. The cholesterol and nicotine-cholesterol group values were not significantly different from one another because of rather large variations between birds. These variations were

greatest at the eight and twelve-week periods when the differences in cholesterol levels and the ratios were most apparent. The effect of nicotine as a hypercholesterolemic or C/P stimulus is at best only suggestive.

The heart and entire aortas were removed from each animal. In each instance the aortas were grossly examined and five sections prepared from the tissues. The first included myocardium, leaflet of mitral valve, and ascending aorta with coronary arteries. The remaining sections consisted of a transverse section of the wall of the aorta, a transverse section of the innominate arteries, a transverse section of the left pulmonary artery, and an opened transverse section of the terminal aorta. In each instance H and E, toluidine blue, and von Kossa's stain were used. In addition, a separate block of the myocardium, aortic valve, coronary artery, and ascending aorta were studied by means of a frozen section with a fat stain.

The gross and microscopic studies of the control and nicotine groups revealed essentially no changes. Three birds of the cholesterol group demonstrated small yellow plaques in the aorta, none of which was greater than 2 mm. in diameter. Upon microscopic examination the plaques were found to consist of slightly thickened intima with a myxomatous, edematous-appearing fibrous tissue forming the main component. No other significant changes were noted in the cholesterol group.

Three birds of the nicotine-cholesterol group also demonstrated plaques in the aorta. Microscopic examination revealed an appreciable increase of fatty material in the thickened intima, particularly in the proximal aorta and aortic valve. There were occasional macrophages filled with a foamy material. The coronary vessels were essentially normal except for minimal thickening.

The fact that minimal pathologic changes observed in both cholesterol groups may indicate that either the particular strain of chicken employed possessed minimal atherogenic susceptibility or that spontaneous regression of the lesions had occurred. Evidence for the latter possibility may be the fall in cholesterol and C/P ratios at eighteen weeks of age. As earlier studies with the rabbit had established a marked effect of nicotine on blood cholesterol levels with a minimum (0.1%) diet of cholesterol, it is possible that the higher dose (1.0%) used in the study obscured any nicotine effect.

REFERENCES

- (1) Hammond, E. C., and Horn, D., *J. Am. Med. Assoc.*, **155**, 1366 (1954).
- (2) Sigler, L. H., *N. Y. State J. Med.*, **55**, 3107 (1955).
- (3) Maslova, K. K., *Byull. Ekspt. Biol. i Med.*, **41**, 420 (1956).
- (4) Wenzel, D. G., and Beckloff, G. L., *THIS JOURNAL*, **47**, 338 (1958).
- (5) Horlick, L., and Katz, N., *Am. Heart J.*, **38**, 336 (1949).

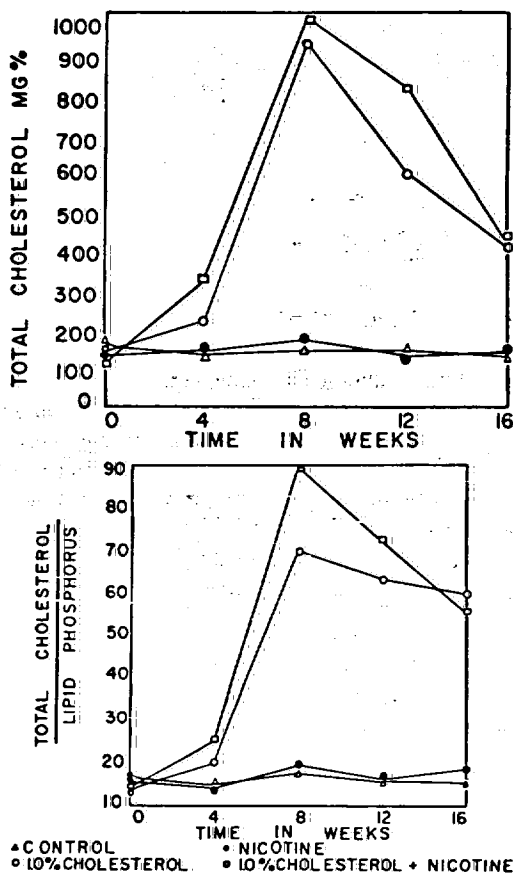


Figure 1.

1003537329

Effect of Nicotine on Cholesterol-Induced
Atherosclerosis in the Rabbit

By DUANE G. WENZEL, Ph.D., JAMES A. TURNER, M.D., AND DONALD KISSI, M.S.

Reprinted from CIRCULATION RESEARCH
Vol. VII, No. 2, March, 1959
Printed in U.S.A.

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1003537331

Effect of Nicotine on Cholesterol-Induced Atherosclerosis in the Rabbit

By DUANE G. WENZEL, PH.D., JAMES A. TURNER, M.D., AND DONALD KISSIL, M.S.

Nicotine was administered orally in three dosage levels to female rabbits fed a 1 per cent cholesterol, 5 per cent oil diet for 24 weeks. Serum cholesterol and phospholipid values and electrocardiograms, both with and without ergonovine stress, were obtained at eight week intervals. Gross and microaortic pathology and cardiac micropathology were determined at the end of the experiment. Serum cholesterol and phospholipid values and aortic atherosclerosis were not affected by the addition of nicotine. Mortality, the incidence of positive ergonovine stress tests, myocardial necrosis and fatty metamorphosis, and peripheral vascular changes were increased. A dose-response relationship could not be established for nicotine.

IT HAS been reported^{1, 2} that nicotine increases the plasma cholesterol levels of male rabbits on a cholesterol-fortified diet. The purpose of this study was to determine the effect of graded doses of nicotine plus a high cholesterol-oil diet on atherosclerosis using the following criteria: serum cholesterol and phospholipid levels, electrocardiographic changes both before and after ergonovine stress, and gross and micropathology of the heart and contiguous vessels.

METHODS

Six groups of 12 albino, New Zealand, six-week old, female rabbits per group were established. All animals weighed between 1.7 and 2.1 Kg. Group 1 received the stock diet of Purina rabbit chow and water ad libitum. For group 2 the food was impregnated with 1 per cent cholesterol and 5 per cent cottonseed oil. Group 3 was fed the stock diet plus the human nicotine equivalent by body weight of two packs of cigarettes daily in the drinking water. This dose is based upon the report that 4 μ g. of nicotine per os produces the same psychic effects as one cigarette in the chronic smoker.³ Using 70 Kg. as the average

adult human weight, the daily two pack equivalent is 2.28 mg. nicotine/Kg. body weight, the one-half pack equivalent is 0.57 mg./Kg., and the one-eighth pack equivalent is 0.142 mg./Kg. Groups 4, 5, and 6 received the group 2 diet and one-eighth, one-half, and two pack equivalents of nicotine respectively. In order to reduce the possibility of acute effects from the nicotine, the dose was gradually increased in order to produce tolerance. Each group was started with one-twelfth the calculated daily dose for the first three days. This amount was increased by a similar quantity every three days until the full dose was being administered on the thirty-sixth day.

Determinations of body weight, serum cholesterol, and phospholipid and electrocardiographic activity both with and without ergonovine were made initially and every eight weeks thereafter for a period of 24 weeks.

Electrocardiography was performed with an Edin C. C.-D. C. amplifier and a Brush Model BL-201 oscillograph at a paper speed of 25 mm./second. The usual limb leads and a chest lead positioned over the heart were used. Electrodes were prepared from silvered hypodermic needles. The rabbits were unanesthetized but were immobilized by use of a rabbit board through which the head could be lowered below the plane of the body. Records were obtained prior to and at 1, 3, 5, and 10 minutes after the injection of 0.05 mg./Kg. ergonovine maleate into the marginal ear vein.

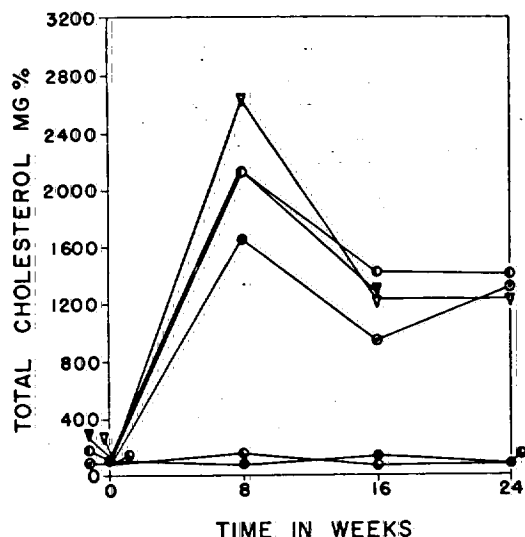
At 24 weeks all surviving animals were killed and in addition to examination for microscopic pathology the degree of aortic sclerosis was grossly graded on a modified 0 to 4 scale. Grade 0 was considered to be less than 2 per cent of the total surface involved, grade 1, up to 10 per cent,

From the Department of Pharmacology, University of Kansas School of Pharmacy and Veterans Administration Hospital, Kansas City, Mo.

Supported by a grant from the Tobacco Industry Research Committee.

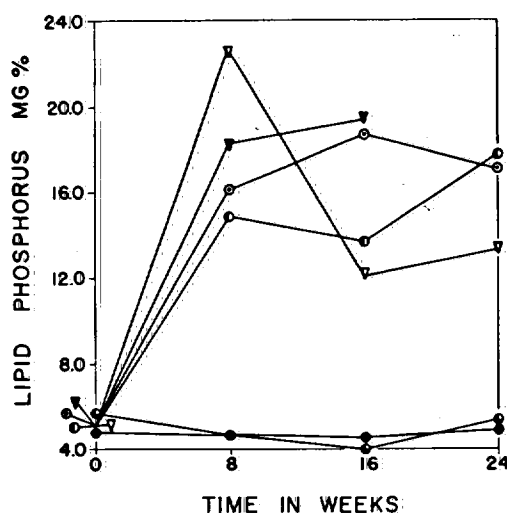
We are indebted to Mr. J. W. Lansdowne, Eli Lilly and Company, for the ergonovine maleate used in this study.

Received for publication September 17, 1958.



- CONTROL
- NICOTINE "2 PACKS"
- 1% CHOLESTEROL
- 1% CHOLESTEROL + NICOTINE "1/8 PACK"
- ▼ 1% CHOLESTEROL + NICOTINE "1/2 PACK"
- ▼ 1% CHOLESTEROL + NICOTINE "2 PACKS"

Fig. 1 Left. Average serum cholesterol values obtained at eight week intervals.



- CONTROL
- NICOTINE "2 PACKS"
- 1% CHOLESTEROL
- 1% CHOLESTEROL + NICOTINE "1/8 PACK"
- ▼ 1% CHOLESTEROL + NICOTINE "1/2 PACK"
- ▼ 1% CHOLESTEROL + NICOTINE "2 PACKS"

Fig. 2 Right. Average phospholipid values obtained at eight week intervals.

grade 2, up to 20 per cent, grade 3, up to 40 per cent, and grade 4, up to 80 per cent. When significant thickening of the lesions were observed, 0.5 was added to the grade. All values were equated to a maximal value of 4 by direct proportion. For the purpose of grading, the aorta was arbitrarily divided into arch, ascending, and thoracic aortic areas. The areas involved were independently estimated by two observers. In borderline cases the dimensions of the plaques were determined by use of a comparator with appropriate reticles.

The heart and aorta were fixed in 10 per cent formalin. Frozen sections were made through the arch of the aorta and the base and apex of the heart. Tissues were stained with oil red O and hematoxylin counterstain.

RESULTS

Serum Cholesterol-Lipid Phosphorus. Figures 1 and 2 illustrate the changes in the serum cholesterol and lipid phosphorus levels. Although an initial linear dose relationship occurs for the nicotine and the serum cholesterol levels, this did not continue, nor was it significant due to large individual variations. Values for the one-half pack group are not given after the sixteenth week because of the

high mortality in this group between 16 and 24 weeks. The 3 nicotine-cholesterol groups showed significantly greater mortalities (70, 91, and 50 per cent) than any of the other groups at 24 weeks (control 8 per cent, nicotine 0, cholesterol 8 per cent). Although there were indications of circulatory failure in those animals who died, correlation of the deaths with cardiac involvement was not attempted.

Ergonovine Stress Test. The electrocardiograms of the cholesterol and nicotine-cholesterol groups did not begin to demonstrate increased abnormalities related to the administration of ergonovine until the sixteenth week. The results as given in table 1 are obtained from the 24 week records. The 16 week records were used for those animals that died between the 16 and 24 week periods. Two criteria of ergonovine stress were selected: A was an ergonovine-induced S-T depression of 1.0 mm. (2 cm. - 1 mv) below the isoelectric level and/or a flattening or inversion of the T wave in leads II, III, or IV. Criterion B was the same as that of A except that flattening of the T wave was eliminated as a posi-

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TABLE 1.—Percentage of Rabbits Exhibiting Positive Ergonovine Stress Test at 24 Weeks

Group no.	Diet	Criterion A* (%)	Criterion B† (%)
1	Control	27.3 (3 of 11)	18.2 (2 of 11)
2	Nicotine	25.0 (3 of 12)	0.0 (0 of 12)
3	Cholesterol	58.3 (7 of 12)	33.3 (4 of 12)
4	Cholesterol plus nicotine "1/8"	71.4 (5 of 7)	71.4 (5 of 7)
5	Cholesterol plus nicotine "1/2"	71.4 (5 of 7)	57.2 (4 of 7)
6	Cholesterol plus nicotine "2"	72.5 (8 of 11)	54.6 (6 of 11)

*Drug-induced depression or elevation of the S-T segment of 1 mm. or more below the isoelectric level and/or "flattening" or inversion of the T wave in lead II, III or IV.

†Same as A with the elimination of T wave "flattening."

tive sign of coronary insufficiency. It was felt that criterion B was more valid, as a number of animals in all groups demonstrated the flattened T wave prior to ergonovine stress. The effect of both pentobarbital anesthesia and the position of the animal on the positivity of the electrocardiogram was determined in a number of cases. It was observed that pentobarbitalization had no effect on positivity, whereas the position, that is, on the back as used in the present study or the side as used by Rinzler,⁴ markedly affected the results. Animals that were negative in the back position were often positive on the side and vice versa. It may be seen from table 1 that a consistently higher percentage of nicotine-cholesterol animals (groups 4 to 6) demonstrated positive electrocardiograms in response to ergonovine than did the cholesterol only group (group 3). Figure 3 illustrates electrocardiograms obtained from a nicotine-cholesterol animal. The typical S-T depression can be seen in the record of the nicotine-cholesterol animal.

Aortic Pathology. Results of the gross grading of the aortas are given in table 2. Rabbits in all groups fed cholesterol exhibited marked involvement of the aortas although there was no difference between the cholesterol

TABLE 2.—Summary of Gross Grading of Rabbit Aortas for Atherosclerosis

Group no.	Diet	Sample size	Ascending aorta	Aortic arch	Thoracic aorta	Mean
1	Control	12	0	.35	.62	.32
2	Nicotine	12	0	0	0	0
3	Cholesterol	12	3.59	4.00	2.33	3.30
4	Cholesterol plus nicotine "1/8"	6	3.47	4.00	1.40	2.95
5	Cholesterol plus nicotine "1/2"	7	3.66	3.92	2.15	3.24
6	Cholesterol plus nicotine "2"	10	3.91	3.95	2.13	3.33

(group 2) and any of the nicotine-cholesterol groups (4 to 6).

Cardiac Micropathology. In the control animals (group 1) there were small amounts of adipose tissue around the coronary vessels. No apparent abnormalities were observed in the walls of any of the blood vessels.

The nicotine group (2) demonstrated minimal paravascular depositions of adipose tissue as well as minimal fatty metamorphosis of myocardial cells. There was, however, in all animals of this group considerable thickening and fibrosis of the small branches of the coronaries.

The cholesterol only group (3) exhibited somewhat varying changes. In general, the coronaries were thickened with advanced atherosclerotic changes. These changes appeared to be most pronounced in the subendocardial vessels. In some arteries closely adjacent to the endocardium, the lumen was reduced in size to approximately one tenth the diameter of the vessel. In 1 animal small areas of necrosis were observed, while 3 animals demonstrated fatty metamorphosis of the myocardial fibers.

The nicotine plus cholesterol groups (4 to 6) exhibited atherosclerotic changes essentially similar to those of the cholesterol only group (3), with certain notable additions. These additions were greater amounts of fatty metamorphosis, and the presence of actual early necrosis of the myocardial tissues in all

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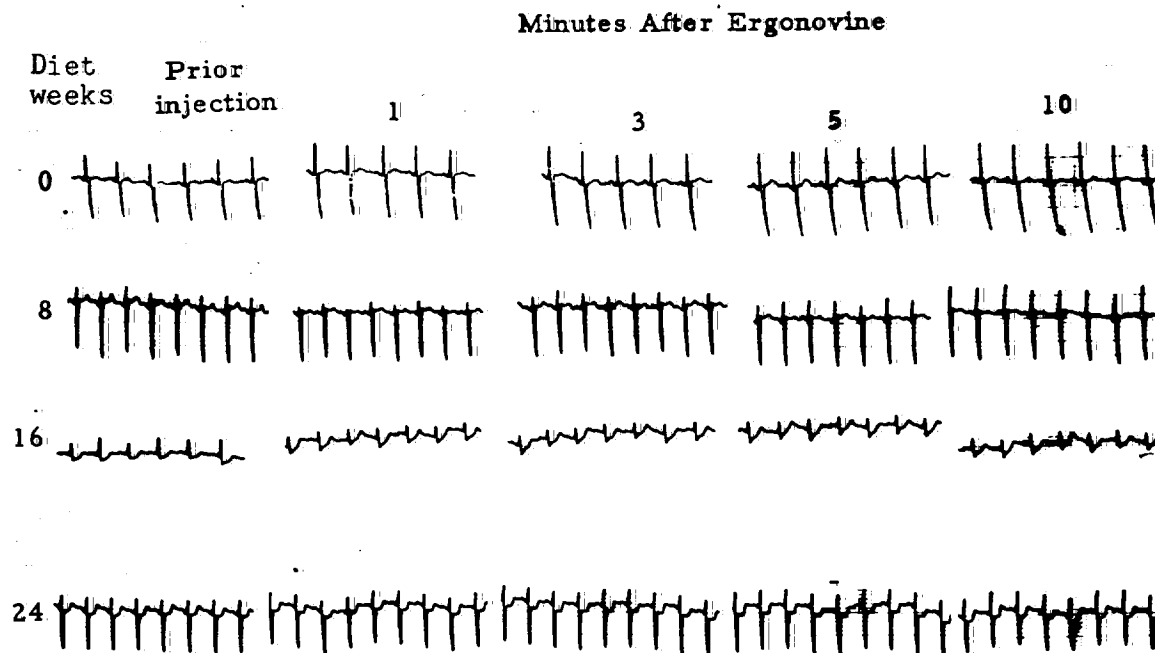


Fig. 3. Serial ergonovine stress tests on a typical positive animal (S-T segment depression) using IV at 25 mm./sec.

animals. Figures 4 and 5 illustrate typical occlusive atherosclerosis of a coronary artery and an area of extensive fatty metamorphosis respectively. An interaction between the nicotine and cholesterol is suggested, as none of the animals fed nicotine only (group 2) demonstrated either fatty metamorphosis or myocardial necrosis. Furthermore, there was no indication from the mortalities or any of the other studies that nicotine given alone produced malignant atherosclerosis.

Peripheral Vascular Changes. Changes in peripheral vascularity appeared in all groups receiving cholesterol. The effect was seen in the limbs as excessive scaling and reddening, followed progressively by gross swelling, alopecia localis, suppurative and sometimes bleeding lesions of all four paws and adjacent areas. These changes appeared first in the nicotine-cholesterol groups (4 to 6) between the 12 and 16 week periods, and progressed rapidly, while in the cholesterol only group (3) the lesions did not appear until after 22 to 23 weeks on the diet, and then progressed only slowly until the termination of the experiment. The fact that these findings were

not seen with the nicotine only group (2) once again suggests an interaction between nicotine and cholesterol.

Several animals receiving cholesterol demonstrated a fatty infiltration of the eye. Not only were scleral deposits observed, as has been previously reported,⁴ but the iris was also markedly infiltrated in some animals. There were no apparently significant differences between the cholesterol and nicotine-cholesterol groups in regard to the eye, and no attempt had been made to note differences in the times of onset.

DISCUSSION

From these studies it appears that an interaction occurred between nicotine and cholesterol in terms of mortality, electrocardiographic and pathologic evidence of coronary atherosclerosis, and peripheral vascular involvement. With one possible exception there was no apparent relationship between the effect of nicotine per se and any of the above criteria. This exception was the consistent production of thickening and fibrosis of the small coronary branches in the nicotine only group (3). Nevertheless, reinforcement of the

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FIG. 4 *Top*. Photomicrograph demonstrating the characteristic occlusive atherosclerosis of a coronary artery in a nicotine-cholesterol animal. Frozen section—Oil-red-O stain. Circa 60 \times .

FIG. 5 *Bottom*. Photomicrograph of a nicotine-cholesterol treated rabbit's heart showing details of extensive fatty metamorphosis bordering on infarction. Frozen section—Oil-red-O stain. Circa 120 \times .

observed cholesterol effects by nicotine were quite apparent. It is interesting that, although a 16 fold nicotine dose differential was employed, no dose-dependent effects were observed.

An earlier study with male rabbits and a 0.1 per cent cholesterol diet demonstrated that nicotine increased total serum cholesterol and phospholipid levels.² This was not confirmed by the present study on female rabbits and a 1 per cent cholesterol plus 5 per cent cottonseed oil diet. One explanation may be that the high cholesterol-oil diet literally flooded the system, thus masking the nicotine effect. It must also be considered that the two rabbit studies also differed in the sex of the animals as well as in the addition of cottonseed oil to the diet in the present study.

While it is not possible to define the mechanism of the interaction between nicotine and cholesterol from the experimental evidence, several points should be considered. As indicated above, the results cannot be attributed to differences in serum cholesterol or phospholipid levels. It may be significant that nicotine apparently did not affect the production of atheroma in the aorta, while evidence of increased atherosclerosis in the heart and periphery was quite apparent. Nicotine is known to exert a rather complex vascular effect in which vasoconstriction predominates. It is possible that prolonged constriction of the vessels involved or other undefined vascular actions of nicotine created the proper physical and/or metabolic environment for the deposition of cholesterol. The observation that the small coronary vessels were thickened and fibrotic in the nicotine only group (2) would strengthen this possibility.

SUMMARY

Groups of female albino rabbits were administered cholesterol, nicotine, and nicotine-cholesterol for a 24 week period. The addition of nicotine to the cholesterol regimen did not significantly affect body weight, serum cholesterol or lipid phosphorus or gross aortic atherosclerosis under the conditions of the test. The nicotine-cholesterol groups demon-

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strated greater mortality, as well as greater electrocardiographic and pathologic evidence of cardiac involvement and peripheral vascular changes, than did the cholesterol or nicotine groups. It is suggested that the combination of nicotine and cholesterol produces a cardiovascular interaction.

SUMMARY IN INTERLINGUA

Grupos de conilias albin recipeva administrationes de (1) cholesterol, (2) nicotina, e (3) nicotina e cholesterol durante periodos de 24 septimanas. Le addition de nicotina al regime de cholesterol non afficeva significativamente le peso corporee, le cholesterol del sero, le phosphoro lipidic del sero, o le apparentia macroscopic del atherosclerosis arterial. In le gruppo recipiente le duo agentes, le mortalitate esseva plus grande, e signos electro-

cardiographic e pathologic indicava plus evidentemente le presentia de affection cardiac e de alterationes periphéro-vascular. Es exprimate le opinion que le combination de nicotina e cholesterol produce un interaction cardiovascular.

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1. MASLOVA, K. K.: Influence of nicotine on experimental atherosclerosis. *Bull. Exper. Biol. Med.* **41**: (420-1-1432) 1956.
2. WENZEL, D. G., AND BECKLOFF, G. L.: The effect of nicotine on experimental hypercholesterolemia in the rabbit. *J. Am. Pharm. Assoc., Sci. Ed.*, **47**: 338, 1958.
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4. RINZLER, S. H., TRAVELL, J., KARP, D., AND CHARLESON, D.: Detection of coronary atherosclerosis in the living rabbit by the ergonovine stress test. *Am. J. Physiol.* **184**: 605, 1956.

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TIRC Grant #155R1

Duane G. Wenzel, Ph.D.
University of Kansas

CONFIDENTIAL

Report No. 3

March 10, 1959

- I. "The Effect of Nicotine on the Thermal Circulation Index, Blood Pressure, Blood Coagulation Time and Cardiac and Peripheral Pathology of Cholesterol-Fed Rabbits."
- II. "The Effect of Nicotine on the Blood Pressure, Blood Coagulation Time and Vascular (Heart, Brain and Periphery) of Atherosclerotic Rats."

- I. Four groups of twelve female rabbits per group have been established as follows:

- Group 1: Control
- Group 2: 1 mg./Kg./day nicotine in drinking water
- Group 3: 1% cholesterol and 5% cottonseed oil in feed
- Group 4: Combined regimens of groups 2 and 3

All animals were approximately six weeks old at the initiation of the experiment. At this time and at four week intervals thereafter the thermal circulation index was determined for each animal. The formula used was:

$$\text{T.C.I.} = \frac{\text{Rectal temperature-skin temperature}}{\text{Skin temperature - room temperature}}$$

Skin and rectal temperatures are obtained by use of thermistor thermometers having an accuracy of $\pm 0.01^{\circ}\text{C}$. Peripheral skin temperature is determined on the shaved dorsal surface of the foot of the hindleg. The mean thermal circulation indices obtained to date along with their standard errors are presented in Table I.

The systolic blood pressures are measured by means of a Grant ear capsule. The mean systolic pressures \pm the standard errors are given in Table II.

Of the original 48 animals six have already died. The deaths occurred in control 1, cholesterol 2, and nicotine-cholesterol 3.

- II. The second study to determine the effect of nicotine upon the atherosclerotic rat has just been started. The groups in this study are essentially the same as with the rabbits except that male Sprague-Dawley strain rats are employed and the atherogenic stimulus consists of the following dietary additives: cholesterol 4%, cholic acid 1%, and thiouracil 0.5%. The rats are being started on this treatment at approximately 100 Gms. wt. Determinations to be conducted include: blood pressure (tail), coagulation time, and the pathology of heart, brain, and periphery (hind legs).

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Table I

Group	0	Time in Weeks		
		4	8	12
Control	4.35 \pm .79	4.15 \pm .23	4.33 \pm .25	4.37 \pm .25
Nicotine	5.25 \pm .41	4.92 \pm .31	4.10 \pm .23	3.74 \pm .16
Cholesterol	3.86 \pm .43	4.99 \pm .35	4.48 \pm .26	4.46 \pm .12
Nicotine-Cholesterol	5.81 \pm .64	5.42 \pm .37	4.15 \pm .19	4.42 \pm .35

Table II

Control	72.00 \pm .92	72.16 \pm .48	74.83 \pm .76	75.33 \pm .89
Nicotine	77.17 \pm 1.06	78.00 \pm 1.29	78.17 \pm 1.32	81.00 \pm 1.69
Cholesterol	78.00 \pm .88	77.33 \pm 1.31	78.10 \pm 1.06	79.27 \pm .73
Nicotine-Cholesterol	76.17 \pm 1.22	76.17 \pm 1.22	78.20 \pm 1.09	86.00 \pm 1.46

EXPENDITURE REPORT
TO MARCH 1, 1959

SALARIES	\$1,158.00
PERMANENT EQUIPMENT	465.00
EXPENDIBLE EQUIPMENT	195.00
OVERHEAD	<u>1,005.00</u>
TOTAL	\$2,823.00

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UNIVERSITY OF KANSAS
THE SCHOOL OF PHARMACY
Lawrence, Kansas

October 3, 1958

Dr. Robert C. Hockett
Associate Scientific Director
Tobacco Industry Research Committee
150 East 42nd Street, New York 17, N. Y.

Dear Doctor Hockett:

Thank you for your recent letter containing your interesting comments concerning the report which I had submitted.

You are right concerning the article in J.A. Ph. A. as this work was the basis of our original request for a grant to T.I.R.C. It was an oversight on my part in not sending reprints of this article and I am enclosing them with this letter. Incidentally, the material submitted in this report (supported by T.I.R.C.) has been accepted by Circulation Research in essentially the same form. A publication date has not yet been set.

Your ideas concerning the mechanism by which nicotine and cholesterol interact are in line with my own thinking on the subject. I have arbitrarily divided the approaches to the problem into three categories.

First, I am interested in determining in greater detail the extent and nature of the atherosclerotic changes. We are now, of course, studying the peripheral manifestations in the rabbit. The rat is also to be used to determine possible species differences. I have in mind another study involving the rat that may make it possible to quantitate cerebrovascular atherosclerosis.

Secondly, the mechanism of the interaction is obviously important and I too have considered among other things the possible role of hypertension. The rat may be particularly useful in such a study. Other approaches could be directed toward the detection of physical changes in the intima or of metabolic changes affecting its functions.

Thirdly, the problem of how to prevent the interaction may be attacked directly by empirical procedures. We have been considering the various aspects of the niacin-nicotine relationship. On several practical and theoretical counts this does appear to be a good possibility for explaining the interaction.

One of my personal problems has been to obtain qualified graduate assistants for these projects and once obtained to provide them with some financial support. The grants which I have received from the Tobacco Industry Research Committee have helped considerably. I am, however, concerned with the planning of long range studies. For example, is it possible to obtain grants with a three-year commitment? This would make it easier

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P. 2 Dr. R. C. Hockett

to obtain the necessary assistants in a keenly competitive market.

I have considered attending the American Public Health meeting in St. Louis, but will probably be unable to come because of a rather confining teaching and research load. Our University policy also makes it rather difficult to attend any meetings other than those at which papers are presented and these requests must be submitted at least one month in advance.

I would enjoy getting together with you should you find time to travel to Lawrence. If this is possible at any time I would be happy to make arrangements for your stay and to pick you up at Kansas City if you come by air.

Sincerely,

/s./

Duane G. Wenzel, Ph. D.
Professor, Pharmacology

DGW/c
Enc.

1003537345

UNIVERSITY OF KANSAS
THE SCHOOL OF PHARMACY
Lawrence, Kansas

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Sincerely,

/s./

Duane G. Wenzel, Ph. D.
Professor, Pharmacology

DGW/c
Enc.

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C O P Y

September 29, 1958

Duane G. Wenzel, Ph.D.
Professor of Pharmacology
The School of Pharmacy
University of Kansas
Lawrence, Kansas

Dear Dr. Wenzel:

We recently ran across your May 1958 article in the Journal of the American Pharmaceutical Association on the Effect of Nicotine on Experimental Hypercholesterolemia in the Rabbit. This was read with considerable interest.

I would assume that this represents the preliminary work you had carried out in this field before receiving the grant from Tobacco Industry Research Committee since it does not contain any acknowledgment to the Committee. It does nevertheless have a very close bearing upon the present project and I think that all members of the Scientific Advisory Board will be glad to have a copy of it along with the copy of your recent progress report which is about to be distributed. Can you supply me with two dozen reprints? We will be glad to defray the cost. We will also want two dozen reprints of any future papers resulting from the project or relating closely to this subject.

Your progress report was read with very real interest and a number of comments and questions come to mind.

In administering nicotine to these rabbits you have had the usual problem of translating human dosages into presumed equivalents for small animals. This has always been a problem and it has become something of a convention to make the adjustment on a weight basis. There are some good arguments for using the relative body surface area instead of weight, on the ground that physiological parameters of many kinds are more closely related to surface than to weight. Some of these arguments are reviewed cogently by Finkel in Cancer Research, 18, 853 (1958), (August issue).

The evidences of interaction between cholesterol and nicotine are, of course, the most interesting observations, and one is tempted to begin speculating on possible mechanisms. I recall the hypothesis that hypertension may be a contributing factor in atherosclerosis in the following manner: It is postulated that (1) the inner wall lining of the blood vessels first loses elasticity through degenerative changes influenced perhaps by heredity, aging, malnutrition or other factors, (2) that hypertension contributes toward development of breaks in this lining by purely mechanical effect, especially in zones of maximum tension and, (3) that plaques tend to be initiated by a process

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Duane G. Wenzel, Ph.D.

September 29, 1958

of filtration of suspended material (lipids?) present in the blood stream through the layers of relatively porous material surrounding the inner lining of the vessels.

If this mechanism has any validity one might assume that nicotine would be most likely to affect stage (1) or stage (2). We know little or nothing about stage (1), but it is common pharmacological knowledge that nicotine can slightly and transiently affect blood pressure possibly by stimulating the release of epinephrine from depots.

At any rate, it might be feasible to determine whether hypertensive drugs in general, and epinephrine particularly, would produce the same kind of effect as nicotine when given chronically in small doses along with cholesterol. This might help determine whether the effect of nicotine is in some way specific, or whether it may be explained wholly as a non-specific effect on the prevailing level of blood pressure.

If the mechanism outlined is valid, one would suppose that the role of cholesterol and associated blood lipids was to provide the filterable material for plaque formation once the necessary conditions of vessel-lining fragility and mechanical rupture had been attained.

In this connection I am reminded of a laboratory agency which is interested in the project of developing a fine instrument capable of measuring the elastic strength of blood vessel linings. Do you think that the development of such an instrument would be useful to studies of the kind in which you are engaged?

The stated hypothesis would explain why plaque formation is not extensive when nicotine is given without cholesterol and extra fat although it would not account for the changes you noted in the smaller coronary branch arteries in your nicotine-only group of animals.

Will you by any chance be attending the American Public Health Association in St. Louis October 26-31? I shall be there for two or three days but probably cannot come to Lawrence at this time.

Sincerely yours,

/s/ Robert C. Hockett
Associate Scientific Director

P.S. For reasons suggested by other research projects, I should be much interested in learning whether extra niacin in the diet of these animals would protect them from the synergistic nicotine and cholesterol effect, or whether a niacin deficiency would augment it.

R.C.H.

cc: McKeen Cattell
R.J. Bing -
Julius H. Comroe

1003537349

The Effect of Nicotine on Experimental Hypercholesterolemia in the Rabbit*

By DUANE G. WENZEL† and GERALD L. BECKLOFF

The administration of 2.28 mg./Kg./day of nicotine alkaloid in the drinking water of rabbits fed a 0.1% cholesterol diet significantly increased the plasma total cholesterol levels over those of control groups. Cholesterol/lipid phosphorus (C/P) ratios, which may be used as an indication of atherogenic susceptibility, were not raised to the same extent because of concomitant increases in lipid phosphorus. Significant differences in serum ascorbic acid, serum stability, bromsulfalein retention, aortic cholesterol content, and aortic plaques could not be demonstrated at terminal determinations possibly because of reduced group sizes.

THE MEDICAL LITERATURE is replete with investigations concerning the effects of nicotine on the cardiovascular system. Clinically it is generally conceded that nicotine should be avoided in vascular diseases such as thromboangiitis obliterans (1), Raynaud's syndrome (2), peripheral arteriosclerosis (3), and its use is inadvisable in the cardiac sensitivity known as tobacco angina (4). These untoward effects of nicotine are probably related to the decreased peripheral blood flow produced by nicotine as demonstrated by skin temperature fall (5), plethysmographic studies (6), and visual observation of capillary blood flow (7).

While it is apparent that the vasoconstrictive effect of nicotine is detrimental to most of the peripheral vascular diseases, it is difficult to relate its action directly to atherosclerosis. It appears to be more or less tacitly assumed that if nicotine is related to atherosclerosis this relationship is not causal in nature but that its action, at most, only aggravates the already existing disease.

Thienes and Butt (8) treating both rats and rabbits with nicotine found an apparent lack of cardiovascular toxicity as a greater percentage of degenerative vascular changes occurred in the controls than in the experimental group. In a clinical study of 301 male diabetics, however, it was reported 53% of the smokers suffered from atherosclerosis as compared to 23% in the nonsmokers (9). Swiss investigators directed attention to the fact that only 6.7% of a large group of coronary patients were nonsmokers as compared to 25.5% of nonsmokers in a comparable control group (10). They further established that there were more heavy smokers among the athero-

sclerotic patients (45%) than the control (28.5%) and the heavier the smoking the younger the age of onset. In a study of 1,520 patients with angina pectoris and coronary thrombosis, Sigler (11) concluded that there was a direct correlation between the amount of smoking and both the early appearance of the first coronary occlusion and the occurrence of death due to this cause. Hammond and Horn (12) in a follow-up study of 190,000 men found that the death rate from coronary artery disease was almost twice as high for men smoking one or more packs of cigarettes daily than for nonsmokers.

As coronary artery disease is usually atherosclerotic in nature it is difficult to account for the increased death rate on the basis of a vasoconstrictive action alone. In fact, smoking has been stated to be of no direct danger to the cardiac patient through coronary vasoconstriction (13). Barger, *et al.* (14), observed that while smoking one cigarette produces an average increase of 19% in coronary blood flow, myocardial oxygen consumption rises by about 27%. In rabbits, nicotine reduces the coronary blood flow in atherosclerotic Langendorff hearts (15).

One possibility is that some of the circumstantial evidence for the role of dietary fats and cholesterol in the production of atherosclerosis may be related in part to nicotine. For example, it has been observed that a steady increase in coronary artery disease occurred in Norway until 1940, the time of the German occupation. During the occupation when the average daily fat intake was reduced from the usual 159 grams to 71 grams, there was essentially a corresponding decrease in deaths due to circulatory diseases. While the probable role of fats in the changing picture is not denied, it must also be considered that during this same period the tobacco consumption was at a relatively low level (16). A similar possibility exists in studies which have related the low fat intake of certain primitive so-

* Received July 10, 1957 from the School of Pharmacy, University of Kansas, Lawrence.

Abstracted from a portion of the data contained in a thesis submitted to the Graduate School of the University of Kansas by Gerald L. Beckloff in partial fulfillment of the requirements for the degree of Master of Science.

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cieties to reduction in cardiovascular disease (17). It is likely that here again the low fat diet is accompanied by minimal tobacco consumption.

The following work was designed as a pilot study to explore the possibility of a direct relationship between nicotine and the plasma cholesterol level. Although the atherosclerotic human does not necessarily have hypercholesterolemia, individuals having evidence of coronary disease as a group tend to have higher than normal serum cholesterol levels (18, 19). Experimentally induced atherosclerosis of animals is usually preceded and (or) accompanied by hypercholesterolemia but can be induced by small doses of cholesterol producing minimal hypercholesterolemia (20).

This initial investigation is limited to the examination of the role of nicotine in total plasma cholesterol and lipid phosphorus levels and to the factors which may be responsible for changes observed.

EXPERIMENTAL

Methods.—Male New Zealand white rabbits were used in this study. The animals were six weeks of age at the beginning of the problem and weighed between 2012 and 2333 grams. Group I, the untreated control, was fed Purina Rabbit Chow Checkers and water *ad libitum*. Group II, the cholesterol control, received the same diet except for the addition of 0.1% cholesterol to the feed. This was added by dissolving the cholesterol in sufficient chloroform to uniformly moisten the pellets during thorough mixing. The chloroform was then removed by evaporation in a steam oven. It should be noted that 0.1% cholesterol in the diet is a smaller quantity than that usually administered. A 1% cholesterol diet is ordinarily considered to produce maximal hypercholesterolemia, but the 0.1% level was selected in order to be able to more readily observe possible increases in the plasma cholesterol level. Group III, the nicotine control, received the control diet plus a measured amount of nicotine alkaloid in the drinking water. The amount of nicotine was calculated to supply a quantity of the alkaloid equivalent on a weight basis to the human consumption of two packs of cigarettes daily. The average daily water consumption per rabbit was 350 ml. Four milligrams of nicotine *per os* has been reported to produce the same psychic effects as smoking one cigarette by a habitual smoker (21). Using 70 Kg. as the average adult human weight, the daily two pack rabbit equivalent is approximately 2.28 mg./Kg. of nicotine alkaloid. This quantity was the final daily amount of nicotine in each 350 ml. drinking water of groups II and IV. All drinking containers were plastic. Group IV had the combined treatments of groups II and III; that is, both cholesterol and nicotine.

In order to reduce the possibility of acute nicotine toxicity, the production of tolerance was attempted by gradually increasing the size of the dose. For the first three days the nicotine equivalent of 3.33

"rabbit cigarettes" was given daily. This same quantity was added to the total daily intake at the end of each three-day period until at the end of thirty-six days the full 40 "rabbit cigarette" equivalent was being administered. The quantity of nicotine was adjusted throughout the experiment to correspond to the increase in body weight.

Immediately prior to the initiation of the study, total plasma cholesterol and lipid phosphorus levels were determined for all animals. These were repeated at intervals of four weeks for the twenty-week test period. Total plasma cholesterol was determined by a modification of the Bloor-Sackett method (22). Plasma lipid phosphorus levels were established by a modified Youngberg procedure (23).

At the termination of the test period additional tests were conducted. A serum ascorbic acid analysis was made of each animal according to Lowry (24). The relative serum stability was determined according to a modification of the work of Ressler, *et al.* (25). Using their technique on hypercholesterolemic serum resulted in spectrophotometric readings too turbid to read. Since the results are simply comparative for the control and experimental groups, the following procedure was used which appeared to allow adequate light transmission. Two-tenths milliliter of 0.006 M zinc acetate solution was added to 1.5 ml. of serum at thirty-second intervals until a total of 1.8 ml. had been added. Two-tenths milliliter of water was then added and readings were made at 510 m μ with a zero setting at 70% transmission. Readings were as low as 21% transmission, yet a number reached over 110% and could not be considered as true numbers. A bromsulphalein test for liver function was conducted according to the usual procedure (26).

When the preceding tests were completed the animals were sacrificed and the aortas graded for gross atherosclerotic lesions by the method of Horlick and Katz (27). After grading, the aortas from the left carotid to the right renal artery were dried over potassium hydroxide, extracted as directed by Faber (28) and the cholesterol determined by the same method as used in the plasma cholesterol determinations. Liver and body weights were also recorded.

RESULTS AND DISCUSSION

Table I and Fig. 1 show that the plasma cholesterol levels were approximately equal for all groups at the beginning of the experiment. While the cholesterol levels of the nicotine and control groups then fell slightly and remained relatively constant throughout the twenty weeks of the experiment, both the cholesterol and nicotine-cholesterol groups had an immediate increase in plasma cholesterol at four weeks with leveling at the eight-week test. The nicotine-cholesterol group, however, had a further increase between the eight- and twelve-week periods with subsequent leveling.

The amount of plasma lipid phosphorus was also approximately the same for all four groups at the onset as seen in Table I and Fig. 2, but the changes differed from those of the plasma cholesterol. The nicotine and control group levels showed a falling trend while both the cholesterol and nicotine-cholesterol rabbits had a rise at four weeks with a fall in the levels of the cholesterol group after this test. The lipid phosphorus values of the nicotine-choles-

Group	
I. Control	
Total Cho	± S.E.
Lipid Ph	± S.E.
C/P Ratio	
II. Choleste	
Total Cho	± S.E.
Lipid Ph	± S.E.
C/P Ratio	
III. Nicotin	
Total Cho	± S.E.
Lipid Ph	± S.E.
C/P Ratio	
IV. Nicotin	
Choleste	
Total Cho	± S.E.
Lipid Ph	± S.E.
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TABLE I.—MEAN TOTAL CHOLESTEROL, LIPID PHOSPHORUS AND C/P RATIOS OF RABBITS*

Group ^b	Time in Weeks					
	0	4	8	12	16	20
I. Control						
Total Cholesterol ± S.E.	60.2 ± 1.7	38.6 ± 3.4	34.7 ± 2.1	34.1 ± 5.7	29.9 ± 3.6	43.4 ± 5.7
Lipid Phosphorus ± S.E.	6.06 ± 0.33	5.63 ± 0.33	4.78 ± 0.24	2.94 ± 0.30	3.25 ± 0.28	3.44 ± 0.23
C/P Ratio ± S.E.	9.93 ± 0.22	6.86 ± 0.28	7.27 ± 0.29	11.6 ± 1.1	9.21 ± 0.69	12.6 ± 1.3
II. Cholesterol						
Total Cholesterol ± S.E.	50.8 ± 5.5	119.7 ± 20.6	89.1 ± 13.8	97.5 ± 14.6	98.8 ± 18.1	100.4 ± 18.9
Lipid Phosphorus ± S.E.	5.94 ± 0.51	7.59 ± 0.67	6.94 ± 0.73	5.22 ± 0.63	4.22 ± 0.38	4.56 ± 0.40
C/P Ratio ± S.E.	8.56 ± 0.27	15.7 ± 1.2	13.6 ± 0.87	18.7 ± 1.3	23.4 ± 2.3	22.3 ± 1.8
III. Nicotine						
Total Cholesterol ± S.E.	70.5 ± 5.1	57.3 ± 6.2	47.3 ± 6.7	54.1 ± 6.3	58.2 ± 13.3	47.6 ± 8.1
Lipid Phosphorus ± S.E.	6.41 ± 0.32	5.41 ± 0.21	6.19 ± 0.27	4.75 ± 0.21	5.25 ± 0.59	4.19 ± 0.43
C/P Ratio ± S.E.	11.2 ± 0.74	10.9 ± 1.5	10.8 ± 0.97	12.2 ± 1.1	10.1 ± 0.91	9.29 ± 0.84
IV. Nicotine + Cholesterol						
Total Cholesterol ± S.E.	64.7 ± 3.9	107.2 ± 15.1	112.3 ± 18.1	195.3 ± 31.7	194.7 ± 27.6	193.1 ± 32.8
Lipid Phosphorus ± S.E.	6.34 ± 0.36	7.38 ± 0.65	7.19 ± 0.82	7.13 ± 0.94	7.44 ± 0.69	8.38 ± 1.03
C/P Ratio ± S.E.	10.1 ± 0.32	13.7 ± 0.92	15.8 ± 2.1	26.1 ± 2.3	25.1 ± 2.0	22.1 ± 2.2

* Total cholesterol and lipid phosphorus are expressed in mg. %.

^b Group I, Control, received stock diet; Group II, Cholesterol, 0.1% cholesterol in diet; Group III, Nicotine, 2.28 mg./Kg. nicotine daily in drinking water; and Group IV, Nicotine-Cholesterol, combined treatments of II and III.

terol group remained relatively constant at the eight- and twelve-week periods but began to increase at sixteen weeks.

The total cholesterol/lipid phosphorus (C/P) ratio is thought by some to be a more sensitive index of atherogenic susceptibility than is the plasma cholesterol level alone (29). Table I and Fig. 3 show that the control and nicotine C/P ratios did vary appreciably but that they are raised in both the cholesterol and nicotine-cholesterol groups. While the nicotine-cholesterol combination produced a faster rise in the ratios than cholesterol alone, the ratios of both groups were approximately equal at twenty weeks. Standard errors are included for each group mean in Table I, but because of inherent wide variations in the cholesterol and lipid phosphorus levels of rabbits (30) more sophisticated statistical approaches were employed. The data were first analyzed for variance by the hierarchic (31) and orthogonal (32) methods. Since significant differences were obtained between groups in both analyses, the two procedures were combined into a regression comparison analysis (33) to determine if the shape of the curves differ significantly from one another.

The *F* values and their significances are given in Table II. The hierarchic analysis of variance reveals significant differences between groups for cholesterol, lipid phosphorus, and the C/P ratios and also between rabbits within groups for cholesterol and lipid phosphorus, but not for the C/P ratios. It may be concluded that there was an overall real difference between the group values although not necessarily for all groups at all times.

The orthogonal analysis of variance also indicates significant differences between the groups and between the periods for the lipid phosphorus and the

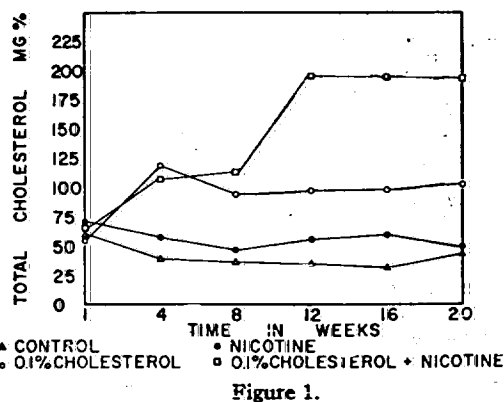


Figure 1.

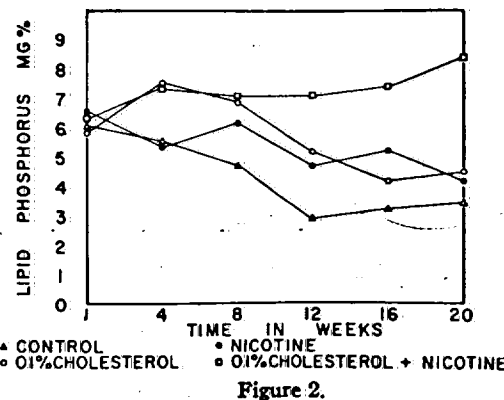


Figure 2.

TABLE II.—ANALYSIS OF VARIANCE

	Hierachic F values	Orthogonal F values
Between groups		
Cholesterol	24.5 ^a	73.6 ^a
Lipid phosphorus	27.9 ^a	28.0 ^a
C/P ratio	45.1 ^a	88.1 ^a
Between rabbits within groups		
Cholesterol	3.16 ^a	..
Lipid phosphorus	3.02 ^a	..
C/P ratio	1.09 ^b	..
Between periods		
Cholesterol	..	1.72 ^b
Lipid phosphorus	..	8.19 ^a
C/P	..	28.61 ^a
Interaction		
Cholesterol	..	5.25 ^a
Lipid phosphorus	..	3.92 ^a
C/P ratio	..	8.43 ^a

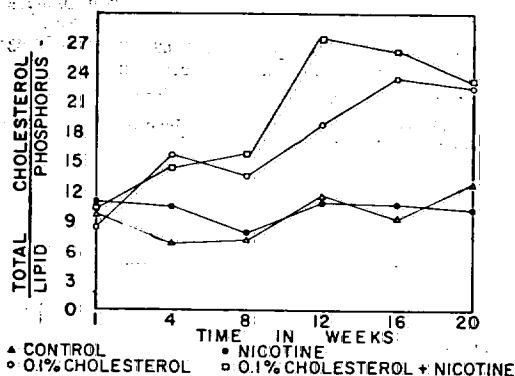
^a P < 0.001.^b Not significant.

Figure 3.

C/P ratios, but not between the periods for cholesterol. This is not to say that there are not significant differences between periods for cholesterol but only that the study as a whole shows no significant differences. This is clarified by the interaction between periods (P) and rabbits (R) which is significant

and demonstrates that real differences do exist between periods but not at all of the times. In other words, as can be seen in Fig. 1, the increases or decreases in plasma cholesterol for all groups did not always proceed in the same direction at one time and there was an overall cancelling effect. Therefore in order to determine whether the nicotine-cholesterol group, for example, had cholesterol values significantly different from those of the control the test period would have to be specified.

Since both the hierachic and orthogonal analyses indicated significant differences between groups, the two procedures were combined into a regression comparison analysis (33) in order to show whether the shapes of the curves differed significantly for the four groups. The results of this analysis are listed in Table III. It can be seen that for several types of curves the differences are significant. This is especially true when they are compared as linear curves.

From the data and its statistical evaluation it may be concluded that while the plasma cholesterol level of the nicotine-cholesterol group was significantly increased by the administration of nicotine over that of the cholesterol group, the lipid phosphorus level of this group also increased. If the C/P ratio is used as an index of atherogenic susceptibility the hypercholesterolemic effect of nicotine is at least partially compensated for by the increase in lipid phosphorus. In order to assess any atherogenic tendency of nicotine it would therefore be necessary to determine the degree of pathological vascular involvement.

The experiment was originally designed to continue for twenty-eight rather than the twenty weeks as shown in the figures and tables. During the last eight weeks a number of animals died in the control, nicotine, and nicotine-cholesterol groups and while values were obtained with the survivors for two more periods they are not included in the data because little reliance could be placed upon the data from small groups. Nine animals were left in the control group, 12 in the cholesterol, five in the nicotine, and four in the nicotine-cholesterol.

At the end of twenty-eight weeks, tests for serum ascorbic acid, serum stability, and bromsulfalein retention were made and the gross aortic lesions, aortic cholesterol, body and liver weights determined

TABLE III.—REGRESSION ANALYSIS OF VARIANCE

Type of Curve	Cholesterol F values				Lipid Phosphorus F values				C/P Ratio F values			
	Control	Cholesterol	Nicotine	Nicotine- Cholesterol	Control	Cholesterol	Nicotine	Nicotine- Cholesterol	Control	Cholesterol	Nicotine	Nicotine- Cholesterol
Linear	18.6 ^c	9.38 ^b	16.0 ^c	52.4 ^c	125.5 ^c	29.8 ^c	26.7 ^c	8.13 ^b	17.3 ^c	209.0 ^c	..	122.1 ^c
Quadratic	19.9 ^c	7.53 ^b	8.21 ^b	7.90 ^b	12.8 ^c	4.58 ^b	19.4 ^c	24.8 ^c	9.74 ^b	23.9 ^c
Cubic	..	8.42 ^b	12.0 ^b	18.4 ^c	20.2 ^c	2.12 ^d	1.71 ^d	3.21 ^d
Quartic	..	5.27 ^a	1.81 ^d	15.9 ^c	25.3 ^c
Quintic	..	2.48 ^d	7.71 ^c	3.70 ^d	6.11 ^a	8.42 ^c	20.1 ^c	9.85 ^c	7.61 ^b

^a < 0.05; ^b P < 0.01; ^c P < 0.001.^d Not significant. Where values are not given whole numbers were not obtained.

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Nicotine	Nicotine- Cholesterol
122.1 ^a	
74 ^b	23.9 ^c
71 ^d	3.21 ^d
85 ^e	7.61 ^b

TABLE IV.—MEAN GROUP VALUES OF TERMINAL DETERMINATIONS

Group	Serum Ascorbic Acid, mg. %	Serum Stability, % Trans- mission	Aortic Total, Cholesterol mg. %	Brom- sulfalein Retention, %	Body Weight, Gm.	Liver Weight, Gm.
Control	0.73	101	11.1	2.2	3910	111
Cholesterol	0.81	78	16.8	6.7	3490	111
Nicotine	0.76	77	30.6	4.1	3561	113
Nicotine-Cholesterol	0.57	96	18.9	5.6	3435	85

on the remaining animals. These values are listed in Table IV. While they cannot be considered to be meaningful because of the reduced number of animals, they are included for reference purposes. Simple analyses of variance were made for all these determinations but no significant differences could be demonstrated. The body weights are of some interest as the nicotine-cholesterol group ate the least and therefore gained the slowest. Rabbits generally develop more arterial lesions if they are well-fed and thriving than when they gain more slowly (30). In this instance the low weight group had the highest plasma cholesterol level.

These tests were performed in an attempt to determine the mechanism by which nicotine produced hypercholesterolemia. It was thought that the action of nicotine might be related to its effect on ascorbic acid as Bourquin (34) found that the level of ascorbic acid in whole blood was lowered as a result of smoking. This observation coupled with the gross and often complete deficiency of ascorbic acid in the arteries of atherosclerotic humans at autopsy and the fact that scurvy in guinea pigs results in rapidly developing atherosclerosis (35) makes the relationship of potential significance. The role of ascorbic acid in atherosclerosis is possibly a function of its control of the synthesis of cholesterol from active acetate (36) and (or) its importance in general vascular health. Serum stability was studied because of the observation by Ressler (25) that the serum of atherosclerotic individuals was relatively unstable, becoming turbid on the addition of certain metallic salts. Liver function was examined because the liver is the principal organ for cholesterol synthesis, turnover, and excretion. In diseases of the liver various alterations of lipid metabolism occur with associated derangements of plasma lipid patterns (37).

Gross examination of the aortas revealed plaques in five of the cholesterol group, three of the nicotine group and one of the nicotine-cholesterol group. According to Katz's (27) system for the quantitative evaluation of atherosclerotic lesions they could be graded as class 1 and 2 involvement. This was then minimal atherosclerosis. Once again the inadequate group sizes made comparison inadvisable.

SUMMARY

1. Four groups of twelve rabbits per group were tested five times at four week intervals for total plasma cholesterol and plasma lipid phosphorus. The groups consisted of a control, 0.1% cholesterol diet, 2.28 mg./Kg./day nicotine alkaloid in the drinking water, and combined cholesterol and nicotine. At twenty-eight weeks, stud-

ies were made of serum ascorbic acid, serum stability, and bromsulfalein retention. Animals were sacrificed and the aortas were examined for gross lesions and their cholesterol content determined. Terminal body and liver weights were also obtained.

2. Tests of significance demonstrated that the administration of nicotine in addition to a cholesterol-containing diet caused significant increases in plasma cholesterol, lipid phosphorus and the C/P ratio of rabbits. The importance of the nicotine enhancement of hypercholesterolemia as an atherogenic stimulus may be negated by the concomitant rise in lipid phosphorus as reflected by the C/P ratios.

3. Significant differences could not be demonstrated in the serum ascorbic acid, serum stability, bromsulfalein retention, and aortic lesions possibly because of the mortality in some groups following the twenty-week period.

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CONFIDENTIAL

TIRC Grant #155

Report No. 2

Duane G. Wenzel, Ph.D.
University of Kansas

July 1, 1957-June 30, 1958

"The Effect of Nicotine on the Blood Cholesterol and
Phospholipids, the Ergonovine-Modified ECG and Vascular
Pathology of Cholesterol-Fed Rabbits."

Experimental Procedure:

Six groups of twelve albino, New Zealand, six-week old, female rabbits per group were established. All animals weighed between 1.7 and 2.1 kilograms. Group I received the stock diet of Purina Rabbit Chow^R and water *ad libitum*. For Group II the food was impregnated with cholesterol 1% and cottonseed oil 5%. Group III was fed the stock diet plus the human nicotine equivalent by body weight of two packs of cigarettes daily in the drinking water. Details of this determination have been reported. Groups IV, V, and VI received the Group II diet and 1/8, 1/2, and 2 "pack equivalents" of nicotine respectively. In order to reduce the possibility of acute effects from the nicotine, the dose was gradually increased in order to produce tolerance.

Determinations of body weight, serum cholesterol and phospholipid and electrocardiographic activity were made initially and every eight weeks thereafter for a period of 24 weeks.

Electrocardiography was performed using an Edin C.C.-D.C. amplifier and a Brush Model BL-201 oscillograph at a paper speed of 25 mm./second. The usual limb leads and a chest lead positioned over the heart were used. Electrodes were prepared from silvered hypodermic needles. The rabbits were unanesthetized but were immobilized by use of a rabbit board through which the head could be lowered below the plane of the body. Records were obtained prior to and at one, three, five and ten minutes following the injection of ergonovine maleate into the marginal ear vein.

At 24 weeks all surviving animals were sacrificed and the degree of aortic sclerosis grossly graded on a modified 0 to 4 scale. Grade 0 was considered to be less than 2 percent of the total surface involved; grade 1, up to 10 percent; grade 2, up to 20 percent; grade 3, up to 40 percent; and grade 4, up to 80 percent. When significant

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thickening of the lesions were observed 0.5 was added to the grade. All values were equated to a maximal value of 4 by direct proportion. For the purpose of grading, the aorta was divided into arch, ascending, and thoracic aortic areas.

The heart and aorta was fixed in ten percent formalin. Tissues were stained with oil-red-O and hematoxylin counterstain and frozen sections made through the arch of the aorta and the base and apex of the heart.

Results:

Body Weight. - The mean body weights of all groups are given in figure 1. Weights within groups were fairly uniform, the standard errors ranging from one to ten percent of the group mean weights. All Cholesterol-fed groups lost weight after the 16-week period.

Serum Cholesterol-Lipid Phosphorus. - Figures 2 and 3 illustrate the changes in the serum cholesterol and lipid phosphorus levels. Although an initial linear dose relationship occurs for the nicotine and the serum cholesterol levels, this did not continue, nor was it significant due to large individual variations. Values for the "1/2 pack" group are not given after the 16th week because of the high mortality in this group between 16 and 24 weeks.

Mortality. - Mortality is shown in figure 4. Most of the animals dying were lost between the 16th and 24th-week test periods. Although there were indications of circulatory failure in those animals who died, it was not attempted to correlate the deaths with cardiac involvement. The nicotine-cholesterol groups demonstrated significantly greater mortalities than any of the other groups ($p < 0.01$ except for Group VI, $p < 0.05$).

Ergonovine Stress Test. - The electrocardiograms of the cholesterol and nicotine-cholesterol groups did not begin to demonstrate increased abnormalities related to the administration of ergonovine until the 16th week. The results as given in Table I are obtained from the 24-week records. Two criteria of ergonovine stress were selected: A was an ergonovine-induced S-T depression of 1.0mm. (2 cm. = 1 mV) below the isoelectric level and (or) a flattening or inversion of the T-wave in leads II, III, or IV. Criterion B was the same as that of A except that flattening of the T-wave was eliminated as a positive sign of coronary insufficiency. It was felt that Criterion A was more valid as a number of animals in all groups demonstrated the

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flattened T-wave prior to ergonovine stress. The effect of both pentobarbital anesthesia and the position of the animal on the positivity of the electrocardiogram was determined in a number of cases. It was observed that pentobarbitalization had no effect on positivity whereas the position, that is on the back as used in the present study or the side as used by Rinzier¹, markedly affected the results. Animals which were negative in the back position were often positive on the side and vice versa. It may be seen from Table I that a consistently higher percentage of nicotine-cholesterol animals (IV-VI) demonstrated positive electrocardiograms in response to ergonovine than did the cholesterol-alone group (II).

Aortic Pathology. - Results of the gross grading of the aortas are given in Table II. Rabbits in all groups fed cholesterol exhibited marked involvement of the aortas although there was no difference between the cholesterol (II) and any of the nicotine-cholesterol groups (IV-VI). Aorta within groups were fairly uniform, the standard

Cardiac Micropathology. - In the control animals (I) there were small amounts of adipose tissue around the coronary vessels. No apparent abnormalities were observed in the walls of any of the blood vessels.

The nicotine group (II) demonstrated minimal paravascular depositions of adipose tissue as well as minimal fatty metamorphosis of myocardial cells. There was, however, in all animals of this group considerable thickening and fibrosis of the small branches of the coronaries.

The cholesterol-only group (III) exhibited somewhat variable changes. In general the coronaries were thickened with advanced atherosclerotic changes. These changes appeared to be most severe in the subendocardial vessels. In some arteries closely adjacent to the endocardium, the lumen was reduced in size to approximately one-tenth the diameter of the vessel. In one animal small areas of necrosis were observed while three animals demonstrated a fatty metamorphosis of the myocardial fibers.

The nicotine-plus-cholesterol groups (IV-VI) exhibited atherosclerotic changes essentially similar to those of the cholesterol-alone group (III) with certain notable additions. These additional findings were greater amounts of fatty metamorphosis and the presence of actual early necrosis of the myocardial tissues in all animals. An interaction between the nicotine and cholesterol is suggested as none of the animals fed nicotine alone (II) demonstrated either fatty metamorphosis or myocardial necrosis. Furthermore there was no indication from

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the mortalities or any of the other studies that nicotine alone produced malignant atherosclerosis.

Peripheral Vascular Changes. - Changes in peripheral vascularity appeared in all groups receiving cholesterol. The effect was seen in the limbs as excessive scaling and reddening followed progressively by gross swelling, alopecia localis, suppurative and sometimes bleeding lesions of all four paws and adjacent areas. These changes appeared first in the nicotine-cholesterol groups (IV-VI) between the 12 and 16-week periods and progressed rapidly while in the cholesterol-alone group (III) the lesions did not appear until 22-23 weeks on the diet and then progressed only slowly until the termination of the experiment. The fact that these findings were not seen with the nicotine-alone group (II) once again suggests an interaction between nicotine and cholesterol.

Several animals receiving cholesterol demonstrated a fatty infiltration of the eye. Not only were scleral deposits observed as has been previously reported¹, but the iris was also markedly infiltrated in some animals. There were no apparently significant differences between the cholesterol and nicotine-cholesterol groups in regard to the eye and no attempt had been made to note differences in the times of onset.

Summary:

Groups of female albino rabbits were administered cholesterol, nicotine, and nicotine-cholesterol for a 24-week period. The addition of nicotine to the cholesterol regimen did not significantly affect body weight, serum cholesterol or lipid phosphorus or gross aortic atherosclerosis under the conditions of the test. The nicotine-cholesterol groups demonstrated greater mortality, as well as greater electrocardiographic and pathologic evidence of cardiac involvement and peripheral vascular changes than did the cholesterol or nicotine groups. It is suggested that the combination of nicotine and cholesterol produces a cardiovascular interaction.

¹RINZLER, S.H., TRAVELL, J., KARP, D., and CHARLSON, D., Detection of coronary atherosclerosis in the living rabbit by the ergonovine stress test, *Am. J. Physiol.*, 184: 605 (1956); and references therein. There was no indication from

Table I. - Percentage of Rabbits Exhibiting a Positive Ergonovine Stress Test at 24 Weeks.

Diet	Criterion A ¹	Criterion B ²
Control	27.3% (3 of 11)	18.2% (2 of 11)
Nicotine	25.0% (3 of 12)	0.0% (0 of 12)
Cholesterol	58.3% (7 of 12)	33.3% (4 of 12)
Cholesterol plus Nicotine "1/8"	71.4% (5 of 7)	71.4% (5 of 7)
Cholesterol plus Nicotine "1/2"	71.4% (5 of 7)	57.2% (4 of 7)
Cholesterol plus Nicotine "2"	72.5% (8 of 11)	54.6% (6 of 11)

¹Drug-induced depression or elevation of the S-T segment of 1.0 mm. or more below the isoelectric level and/or "flattening" or inversion of the T-wave in lead II, III or IV.

²Same as A with the elimination of T-wave "flattening."

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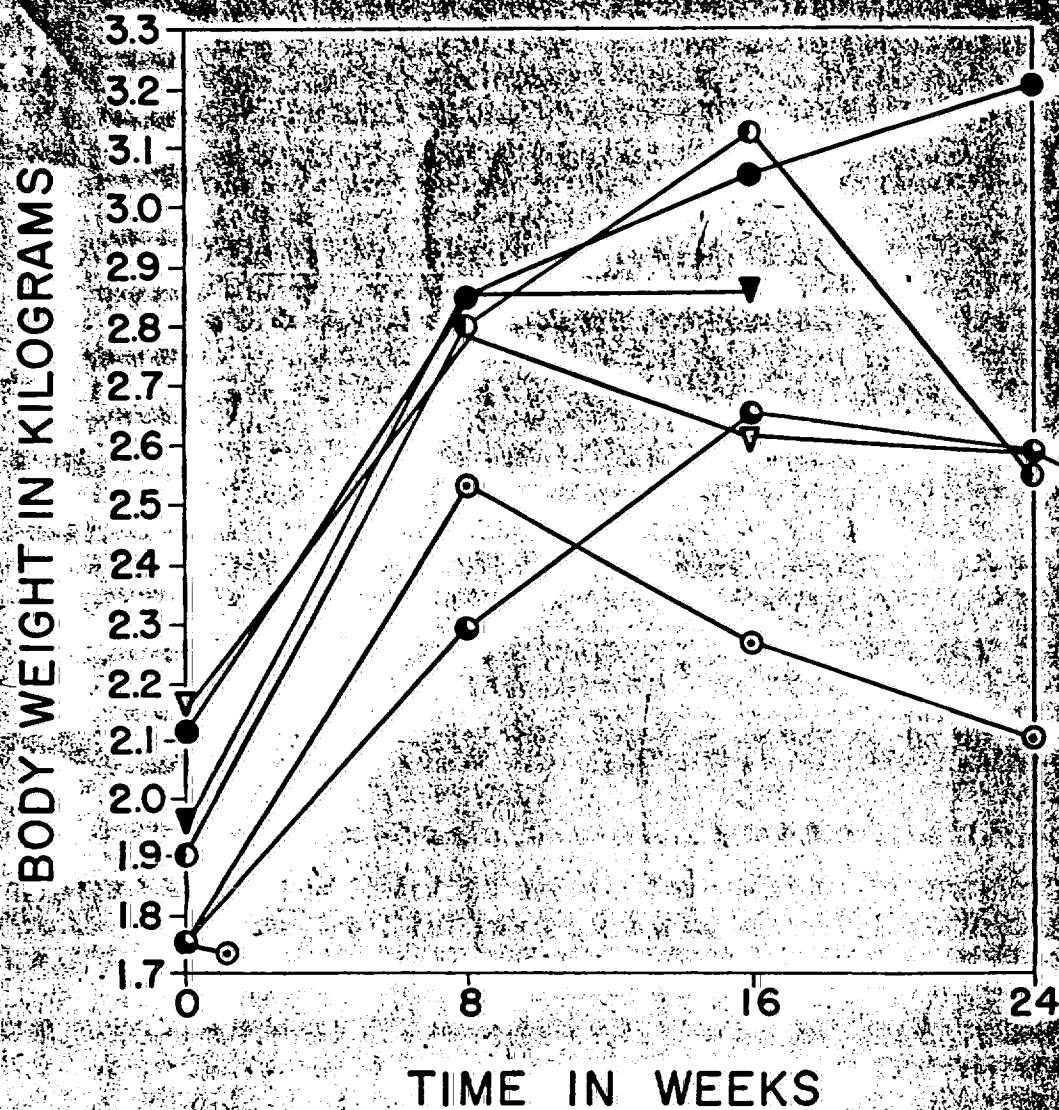
Table II. - Summary of the Gross Grading of the Rabbit Aortas for Atherosclerosis.

Groups	Sample Size	Ascending Aorta	Aortic Arch	Thoracic Aorta	Mean
Control (I)	12	0	.35	.62	.32
Nicotine (II)	12	0	0	0	0
Cholesterol (III)	12	3.59	4.00	2.33	3.30
Cholesterol plus Nicotine "1/8" (IV)	6	3.47	4.00	1.40	2.95
Cholesterol plus Nicotine "1/2" (V)	7	3.66	3.92	2.15	3.24
Cholesterol plus Nicotine "2" (VI)	10	3.91	3.95	2.13	3.33

1. See text for description of the grading scale used.

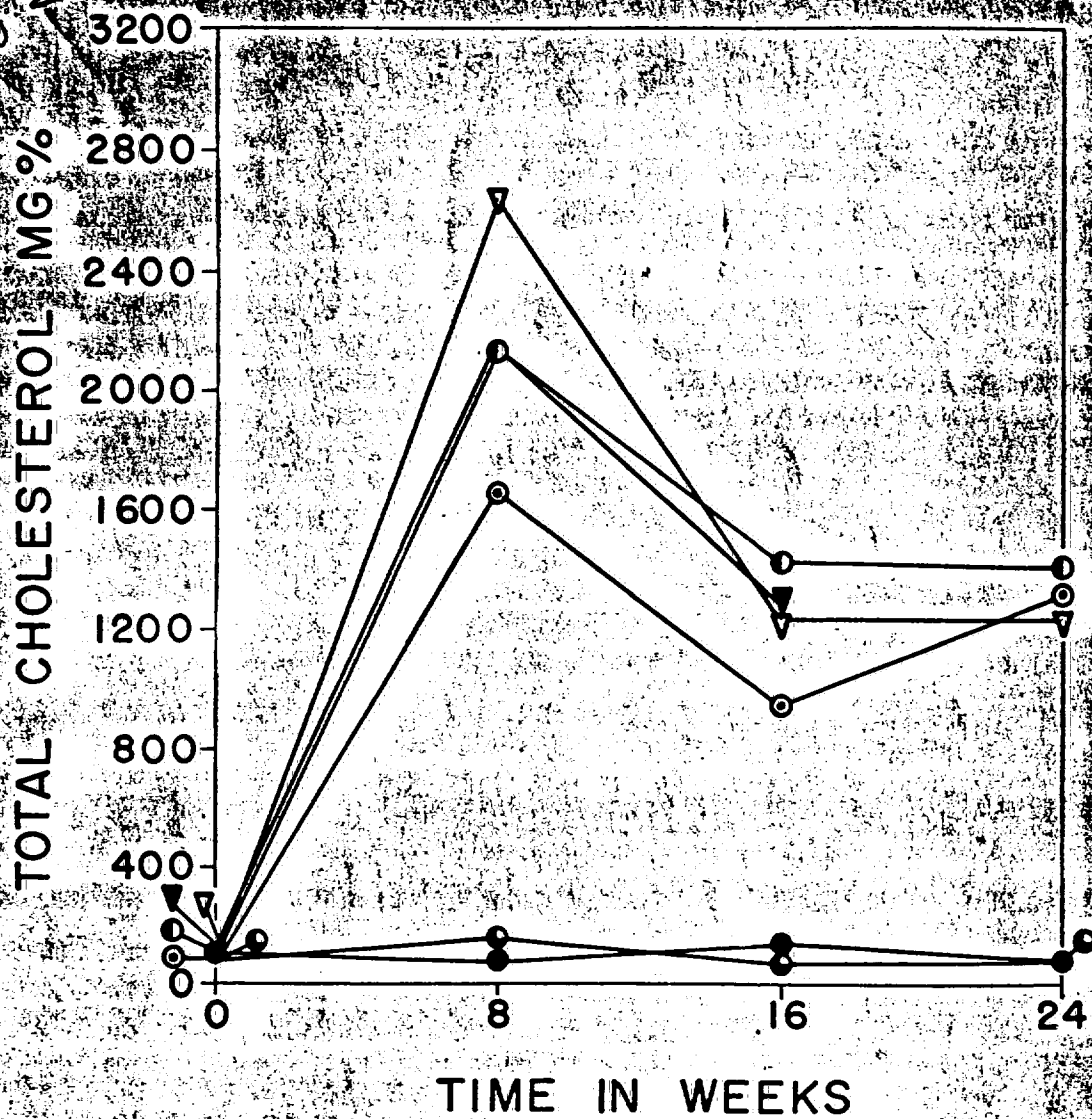
Same as A with the elimination of T-wave flattening.

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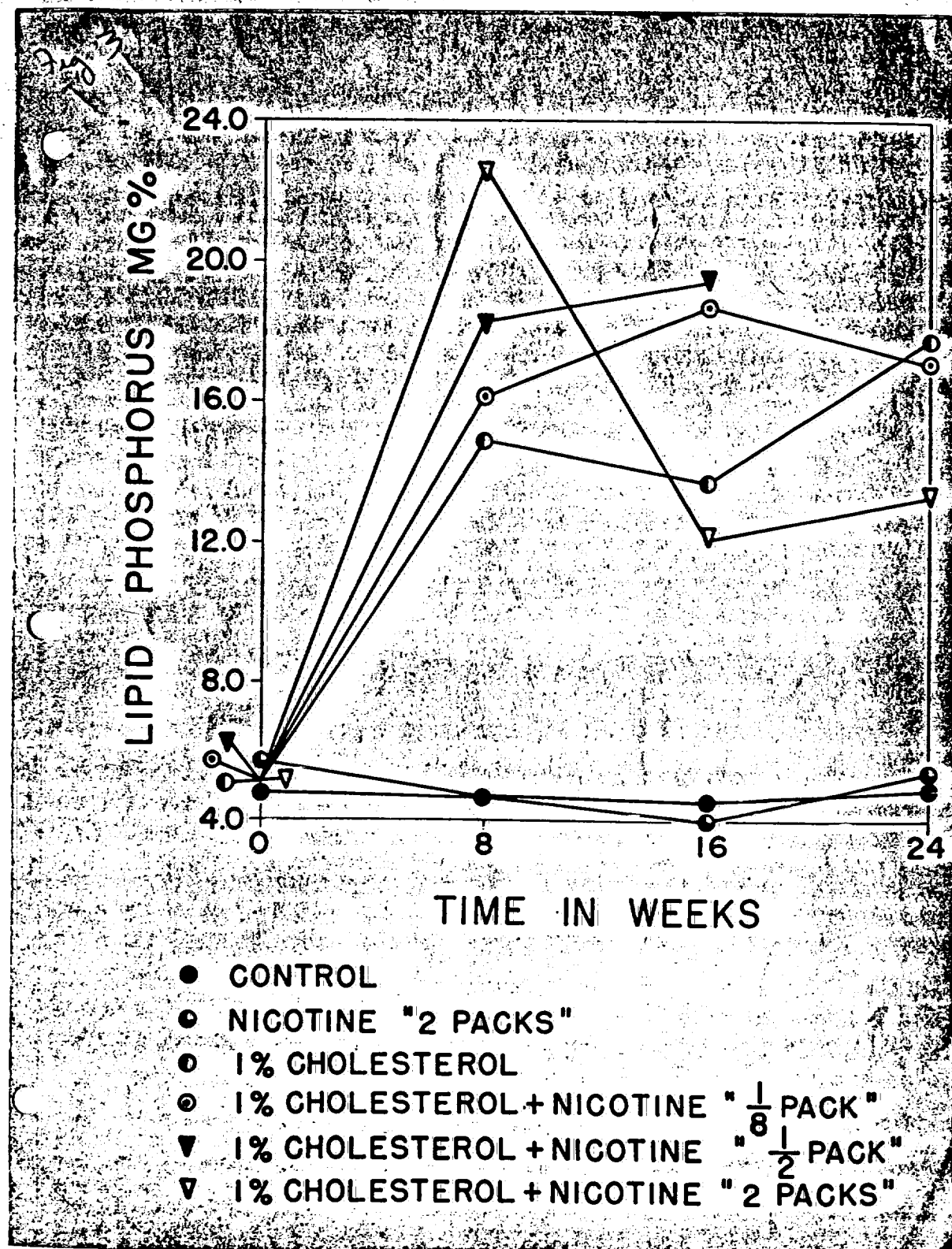
- CONTROL
- NICOTINE "2 PACKS"
- 1% CHOLESTEROL
- 1% CHOLESTEROL + NICOTINE " $\frac{1}{8}$ PACK"
- ▼ 1% CHOLESTEROL + NICOTINE " $\frac{1}{2}$ PACK"
- ▼ 1% CHOLESTEROL + NICOTINE "2 PACKS"

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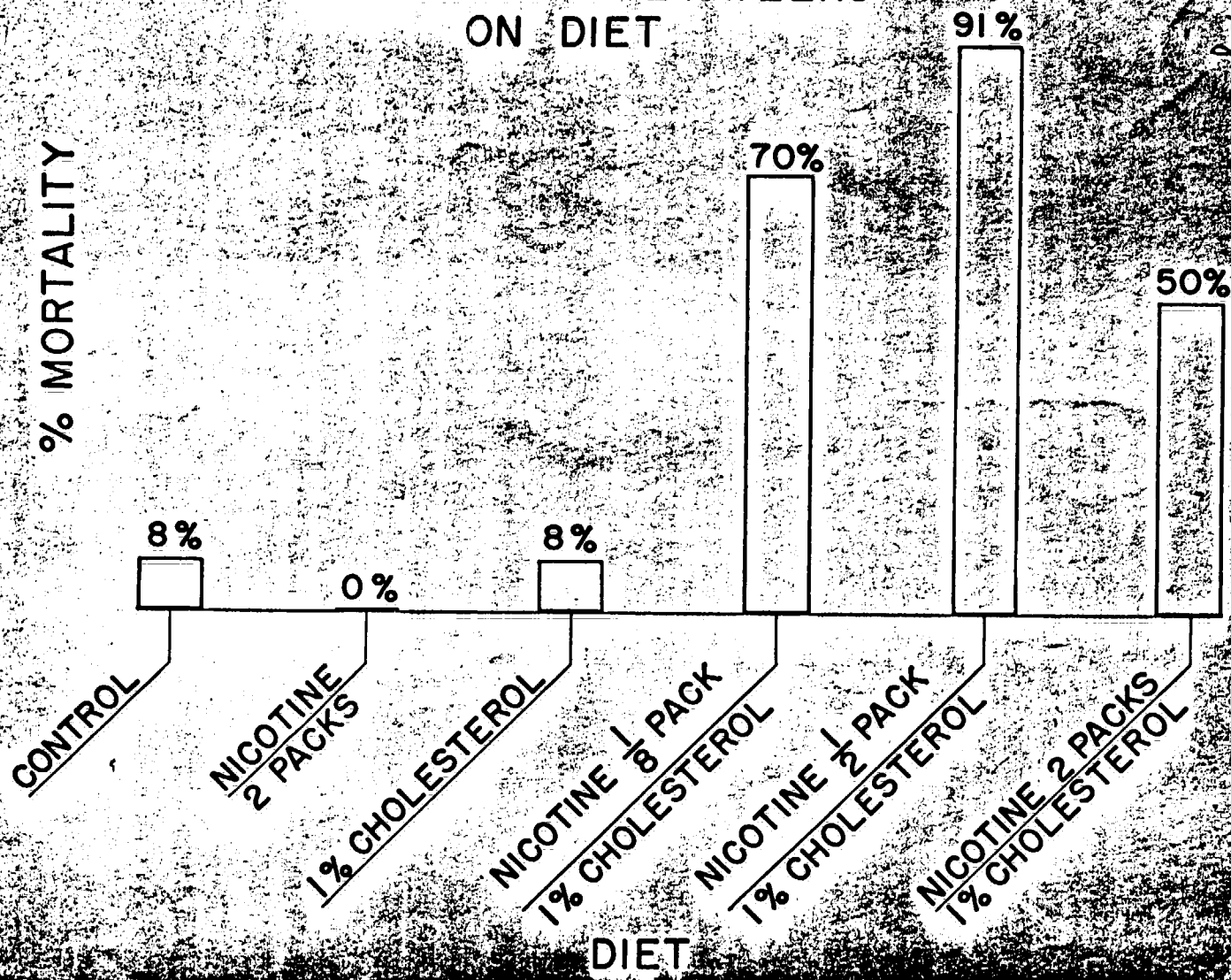
- CONTROL
- NICOTINE "2 PACKS"
- 1% CHOLESTEROL
- ⊙ 1% CHOLESTEROL + NICOTINE " $\frac{1}{8}$ PACK"
- ▼ 1% CHOLESTEROL + NICOTINE " $\frac{1}{2}$ PACK"
- ▼ 1% CHOLESTEROL + NICOTINE "2 PACKS"

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MORTALITY AT 24 WEEKS
ON DIET



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TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET
NEW YORK 17, N.Y.

#155 RL
(Activated 7/1/57)

Application For Research Grant

Date: April 14, 1958

1. Name of Investigator: **Duane G. Wenzel, Ph.D.**
2. Title: **Professor of Pharmacology**
3. Institution & Address: **School of Pharmacy
University of Kansas
Lawrence, Kansas**
4. Project or Subject: **Further studies of the biological interaction between nicotine and hypercholesterolemia.**

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

The proposed study is to consist of two parts based upon observations made in prior work of the applicant. The observations related to the first proposal are as follows:

(a) Rabbits treated with a hypercholesterolemic diet plus nicotine (at all dosage levels) developed signs of peripheral vascular disturbances. These were manifest as reddening, alopecia localis, swelling and the development of severe lesions on all four paws. The symptoms progressed in the order named and were observed in three experimental groups receiving both nicotine and cholesterol, but were not observed in the control, cholesterol, or nicotine groups.

(b) The eyes of most of the cholesterol-nicotine animals developed cholesterol infiltration in both the sclera and the iris. The same effect, but apparently of a somewhat lesser intensity, was also observed with the cholesterol-only group.

On the basis of these two observations it is felt that the eye may be useful for the evaluation of the early deposition of cholesterol both in the sclera and iris and in the eyeground. It is anticipated that the time of onset and degree of involvement might be used to determine possible differences between the effects of hypercholesterolemia alone and with added nicotine. An ophthalmological camera is available, if necessary, to document any retinal effects. Three groups of female rabbits will be used for this study, a high cholesterol, a nicotine, and a nicotine-cholesterol group. To evaluate possible peripheral vascular disturbances the skin temperature of the extremities and that of the rectum will be measured with a thermistor thermometer to determine the thermal circulation index.

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It was further observed that the mortality of the hypercholesterolemic-nicotine-treated rabbits was significantly greater in two separate studies than that of the hypercholesterolemic or nicotine groups. These latter groups were essentially intact at the conclusion of the studies while the majority of the nicotine-hypercholesterolemic animals were dead. This lethal interaction was not readily apparent with chickens.

It is proposed that rats be used to determine the possible species relationship of this effect and the resultant pathology. Three groups of male rats are to be used as in the other portion of the proposal relating to rabbits. The groups of rats are to be at least 20 each and are to be housed in separate cages. Hypercholesterolemia will be induced according to the procedure of Vitale et al. (1) The pathology will be determined by Dr. James Turner.

The amount of nicotine to be used in both studies will be the equivalent of two packs of cigarettes daily in terms of average nicotine absorption by man and body weight. This amount per se in previous studies with rabbits and chickens was found to have no demonstrable effect on the mortality rate or pathology.

(1) Vitale, J.J., White, F.L., Hakumura, M., Hegsted, D.M., Zarecheck, H., and Hellerstein, E.K., J. Exp. Med., 106, 757 (1957)

1003537369

6. Budget Plan:

Salaries	5,200.00
Expendable Supplies	600.00
Permanent Equipment	700.00
Overhead	1,005.00
Other (15%)	200.00
(Travel)	
Total	7,705.00

As expenses will be greatest at the initiation of the work, it is requested that the amount be made available immediately rather than in quarterly payments.

Duration of Work:

One year, July 1, 1958 through June 30, 1959

An air-conditioned animal room and completely equipped research laboratories are available for this project. The project director will be available on a full-time basis for two months of the summer and on a part-time basis during the remainder of the year. A one-half time research associate will work on the project throughout the year. Other personnel whose services will be used include: a pathologist, laboratory technician, animal room caretaker, biometrician and others. Specialized equipment in the Department of Pharmacology includes a Warburg gasometer apparatus, spectrophotometer, oscilloscope and recorder, oscillograph, amplifiers, preamplifiers, stimulators, flame photometer, etc.

None

10. Additional Information (Including relation of work to other projects and other sources of supply):

The proposed study is based upon results of a previous experiment with rabbits to be published in the J. Am. Pharm. Assoc., Sci. Ed. March, 1958; a similar project completed with chickens; and a project using rabbits and now being supported by the Tobacco Industry Research Committee.

Signature /s/ Duane G. Wenzel
Director of Project

/s/ William J. Averisinger, Jr.
Business Officer of the Institution
Associate Dean, Graduate School

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Application For Research Grant

Date: February 22, 1955

1. Name of Investigator:

Duane G. Wenzel

2. Title:

Associate Professor of Pharmacology

3. Institution

& Address:

School of Pharmacy, University of Kansas, Lawrence, Kansas

4. Project or Subject:

The Effect of Chronic Cigarette Smoke Inhalation on Experimental Atherosclerosis of the Rabbit.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Four experimental groups of rabbits are to be maintained for approximately one year as follows:

Group I, fed threshold-atherosclerotic levels of cholesterol and administered cigarette smoke daily to roughly correspond to the "heavy" human smoker;

Group II, fed a normal diet plus smoking as in Group I;

Group III, fed threshold-atherosclerotic levels of cholesterol, and

Group IV, fed a normal diet.

Each group is to have regular determinations made of the serum cholesterol/phospholipid ratio throughout the year. At the end of the experimental period, the animals are to be sacrificed and the following determined:

1. Histology of the cardiac coronary vessels;
2. Transverse and longitudinal distensibility of the aorta; and
3. Cholesterol and calcium content of the aorta.

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6. Budget Plan:

Salaries	\$3,200.00
Expendable Supplies	450.00
Permanent Equipment	800.00
Overhead 8%	356.00
Other	
Total	\$4,806.00

7. Anticipated Duration of Work: One year, July 1, 1955 through June 30, 1956. If, however, significant differences are found between the control and tobacco-smoking groups, further studies will be desirable.

8. Facilities and Staff Available:

A new air-conditioned animal room and research laboratories with all types of pharmacological and chemical equipment are available.

The staff available for this project consists of the Project Director (DGN), a research assistant in pharmacology and whatever additional graduate assistants are necessary. The Project Director will be able to spend full time for two months (July and August, 1955) in setting up and starting the project. The remainder of the year part of his time will be used to direct and supervise the project.

9. Additional Requirements:

None

10. Additional Information (Including relation of work to other projects and other sources of supply):

It is anticipated that this project will aid in confirming or denying reports implicating smoking as a cause of coronary disease (1). The chronic production of atherosclerosis is hoped to give a condition which will closely resemble the human form of the disease (2) and will thus aid in establishing the role of smoking in atherosclerosis.

(1.) Hammond, E. C., J.A. Ph. A., Pract. Ed., 15, 537 (1954).

(2.) Duff, G. L. and McMullan, G. C., Am. J. Med., 11, 92 (1951).

Signature /s/ Duane G. Wenzel
Director of Project

Duane G. Wenzel

/s/ William J. Argersinger, Jr.
Business Officer of the Institution

Wkk W. J. Argersinger, Jr.
Assistant Dean, Graduate School

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NO CHARTS ATTACHED

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

Application For Research Grant

#155

Date: March 29, 1957

1. Name of Investigator:

Duane G. Wenzel

2. Title:

Professor of Pharmacology

3. Institution

& Address:

School of Pharmacy, University of Kansas, Lawrence, Kansas

4. Project or Subject:

The determination of the chronic effects of orally administered nicotine on serum cholesterol and phospholipids; the electrocardiographic response to ergonovine; and the vascular pathology of cholesterol-fed rabbits.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Prior Work On This Project The accompanying sheet and graphs summarize the completed pilot study. In brief, the daily administration of 2 mg./Kg. of nicotine in the drinking water of male rabbits fed 0.1% cholesterol in their diets produced a significant increase in the blood cholesterol levels. Work is currently underway using essentially the same experimental procedure with chickens instead of rabbits.

It is proposed that the rabbit study be repeated with certain additions and modifications in order to determine if nicotine increases vascular pathology as well as serum cholesterol levels.

First, female rabbits are to be used instead of male as some male rabbits are refractory to the development of atherosclerosis (1).

Second, the period of study will be extended beyond that of the pilot study as rabbits must be close to one year of age and be kept on a cholesterol diet for at least twenty weeks before vascular pathology appears (2,3).

Third, several levels of nicotine are to be administered in order to obtain evidence either for or against a causal relationship between nicotine and hypercholesterolemia as well as the possible relationship between nicotine and vascular pathology. Doses of 0.125 mg./Kg., 0.5 mg./Kg. and 2.0 mg./Kg. are to be administered to separate groups. The doses were selected on the basis of the report that 4 mg. of nicotine orally in man causes similar psychic effects as smoking one cigarette (4). On a weight basis, a dose of 1.0 mg./Kg. of nicotine represents approximately the equivalent of one package of cigarettes.

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Finally, cardiovascular pathology is to be sought first of all in the living animals throughout the experiment by means of electrocardiographs under ergonovine stress (3). This technique should reveal whether atherosclerosis is demonstrable in the nicotine-cholesterol group prior to the cholesterol group. Vascular pathology will also be studied according to standard histological techniques. This phase of the work will be under the supervision of Dr. Harlan I. Firminger, Professor of Pathology, University of Kansas Medical School, Kansas City, Kansas.

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6. Budget Plan:

Salaries	4,654.00
Expendable Supplies	600.00
Permanent Equipment	785.00
Overhead 15%	905.00
Other	
Total	6,944.00

As expenses will be greatest at the initiation of the work, it is requested that at least 1/2 the amount be made available immediately rather than in quarterly payments.

7. Anticipated Duration of Work:

July 1, 1957 through June 30, 1958.

8. Facilities and Staff Available:

An air-conditioned animal room and completely equipped research laboratories are available for this project. The project director will be available on a full-time basis for two months of the summer and on a part-time basis during the remainder of the year. A one-half time research assistant will work on the project throughout the year. Other personnel whose services will be used include a pathologist, laboratory technician and animal room caretaker.

9. Additional Equipment: Equipment in the Department of Pharmacology includes a Warburg Manometer, oscilloscope and recorder, flame photometer etc.

None.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The studies with nicotine and atherosclerosis to date have been paid for primarily by the University of Kansas, however, we are unable to receive further support because of the heavy costs involved.

Keith L. Nitcher
Comptroller
121 Strong Hall
University of Kansas

Signature Dr. Duane G. Wenzel
Director of Project

W. J. Graessinger, Jr.
Business Officer of the Institution

Assistant Dean, Graduate School

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PREVIOUS WORK DONE ON THIS PROJECT

Albino, New Zealand, male rabbits were the experimental animals in the study just completed. At six weeks of age four groups of twelve animals per group were arranged as follows:

- Group 1. Control-----Fed Purina Rabbit Chow and water ad libitum.
- Group 2. Cholesterol-----0.1% cholesterol in above diet.
- Group 3. Nicotine-----2 mg./Kg. nicotine daily administered in drinking water.
- Group 4. Nicotine-Cholesterol-----Combined procedures of groups 2 and 3.

At the onset of the experiment and at monthly intervals thereafter for five months, determinations were made of the total plasma cholesterol and lipid phosphorous. The following values were obtained:

Group	Time in Weeks					
	Total cholesterol/Lipid phosphorous (mg.%) ¹					
	0	4	8	12	16	20
Control	$\frac{60}{6}$	$\frac{39}{6}$	$\frac{35}{5}$	$\frac{34}{3}$	$\frac{30}{3}$	$\frac{43}{3}$
Cholesterol	$\frac{51}{6}$	$\frac{120}{8}$	$\frac{95}{7}$	$\frac{97}{5}$	$\frac{99}{4}$	$\frac{102}{5}$
Nicotine	$\frac{70}{6}$	$\frac{57}{5}$	$\frac{47}{6}$	$\frac{54}{5}$	$\frac{58}{5}$	$\frac{47}{4}$
Cholesterol / nicotine	$\frac{64}{6}$	$\frac{107}{7}$	$\frac{112}{7}$	$\frac{195}{7}$	$\frac{195}{7}$	$\frac{193}{8}$

¹ For convenience of comparison values are given in round numbers.

At twenty weeks of age blood levels of vitamin C were determined because of reports that nicotine lowers the blood level of this vitamin. No significant differences were observed between the control and nicotine groups. Studies were also made at this time for the relative serum stability as determined by the turbidity produced upon the addition of a zinc salt. Once again no significant differences were observed. Because of the possible role of the liver in hypercholesterolemia, bromsulphalein was used to evaluate liver function. No conclusive differences were established.

Upon sacrifice a gross examination of the aortas did not indicate any greater incidence of plaques in the experimental animals than in the controls. Microscopic pathology was not determined because of the lack of a qualified pathologist at this time. In the proposed studies to follow, however, the services of Dr. Harlan T. Firminger, Professor of Pathology, are available.

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Duane G. Wenzel

Biography

Born, September 18, 1920.

B.S. Pharmacy, University of Wisconsin 1942.

Ph.D. Pharmacology and Pharmaceutical Chemistry 1948, University of Wisconsin.

Assistant Professor of Pharmacology, School of Pharmacy, U. of Kansas 1948.

Associate Professor of Pharmacology, School of Pharmacy, U. of Kansas 1952.

Professor of Pharmacology, School of Pharmacy, U. of Kansas 1956.

Associate editor, *Midwestern Druggist*, 1948 -----.

Committee on Physiological Testing, American Pharmaceutical Association, 1956-59.

Scientific Bibliography

Actions of Podophyllin Derivatives, *Fed. Proc.*, 7, 1 (1948).

A Study of the Toxicity and Anthelmintic Activity of n-Butylidene Chloride, *J. Pharm. Pharmacol.*, 3, 169 (1951).

Osageorange Oil, *Trans. Kans. Acad. Sci.*, 54, 94 (1951).

Pharmacological Actions of *Paeonia officinalis*, *J. Am. Pharm. Assoc.*, 41, 162 (1952).

The Effect of Antipyretics on the Erythrocytic Sedimentation Rate of Rats, *J. Am. Pharm. Assoc.*, 42, 600 (1953).

Cardiac Activity of Unsaturated Lactones as Related to Their Theoretical Peroxide Formation, *J. Am. Pharm. Assoc.*, 42, 653 (1953).

Central Depressant Properties of Uracil and Related Oxypyrimidines, *J. Am. Pharm. Assoc.*, 44, 56 (1955).

Anticonvulsant Activity of Some Uracils and Related Compounds, 44, 550 (1955).

The Evaluation of Arsenic as an Antiasthmatic I. Effect on Experimental Asthma of Guinea Pigs, *J. Am. Pharm. Assoc.*, 45, 1 (1956).

The Evaluation of Arsenic as an Antiasthmatic II. A study of Possible Mechanisms, *J. Am. Pharm. Assoc.*, 45, 6 (1956).

An Investigation of Triterpenes as Steroid Hormones, *J. Am. Pharm. Assoc.*, 45, 284 (1956).

Estrogenic Activity of Some Flavonoids, *J. Am. Pharm. Assoc.*, 45, 367 (1956).

The Effect of Triterpenes on the Excretion of Sodium and Potassium by Rats, *J. Am. Pharm. Assoc.*, 45, 372 (1956).

Antispasmodic and Related Pharmacological Actions of Some γ -Arylpropylamines, *J. Am. Pharm. Assoc.*, 45, 414, (1956).

Duane G. Wenzel

(2)

Scientific Bibliography (Cont.)

Anticonvulsant Properties of Some Alkyldiols, Alkyldiones and Related Compounds, J. Am. Pharm. Assoc., 45, 669 (1956).

Central Stimulating Properties of Some Terpenones, J. Am. Pharm. Assoc., 46, 77 (1957).

Studies Completed or in Press

The Effect of Nicotine on Experimental Hypercholesterolemia in the Rabbit

The Antiveratrinic Action of Some Simple Lactones and Peroxides

The Effect of BAL on the Amine Oxidase/Decarboxylase Ratio of the Kidneys of Normal and Renal Hypertensive Rats

The Relationship of Electroshock and Anticonvulsants to Brain Succinic Dehydrogenase

Anthelmintic Activity of Peroxide-Forming Compounds

Pharmacological Actions of Optical Isomers of Some Phosphonamidates

at

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TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street New York 17, N. Y.

Application for Research Grant

Date: July 10, 1959

1. Name of Investigator: Frederick E. Whiskin, M.D., C.M.
2. Title: Director, Division of Health and Personality
Equilibrium
3. Institution: The Age Center of New England, Inc.
& Address: 160 Commonwealth Avenue
Boston 16, Massachusetts
4. Project or Subject: Proposal for a pilot study of the smoking habits
of Age Center members.
5. Detailed Plan of Procedure:

Objective

It is proposed to study the smoking habits of The Age Center members in relation to a large amount of medical, psychological and demographic data which have already been gathered on these members. We propose to divide our sample on the basis of a new questionnaire to be prepared for this study between heavy smokers, moderate smokers and non-smokers, both male and female. We will then relate by statistical techniques appropriate to the sample under consideration the other data which have already been gathered on the extremes of various dimensions.

Method

Our work will be divided into three overlapping parts. First, we will develop a questionnaire on smoking habits and study the literature for hypotheses pertinent to this project. Second, we will sort and relate data. Third, we will write a report of our findings and develop new hypotheses for further work.

We have studied questionnaires on smoking habits used by other groups. Each of them seems to have some limitations for our purposes. We feel we can obtain greater refinement and selectivity in a new questionnaire which we will mail to our members. We then expect to follow up this mailing with face-to-face interviews with a randomly selected group of heavy smokers and non-smokers who reply in order to make certain of the validity of the responses.

While we are waiting for the questionnaires to be returned from our members, we will undertake a review of the literature with special reference to the hypotheses which might have significance in relation to our special subject population. As this is completed, it is expected that we will develop other hypotheses which can be readily tested at this point and may indicate the directions of further research. It is expected that this foundation will be completed sometime after the middle of August.

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The second stage of the study is essentially one of clerical and mechanical processing of the data in relation to ideas to be developed in the first stage. We are purposely omitting any discussion of the notions that are now in our thinking since they should change materially during the next few weeks. Since we have substantial medical history and psychological appraisal on our members, we expect to generally explore the relation of their various elements to smoking habits. Perhaps a word of caution would be advisable. Since our sample excludes those who are now in serious health problems, the hypotheses developed will be limited to those in which history of health disabilities is important rather than current illnesses.

The third and final stage will involve numerous staff conferences with those interested in the project in order to extract all possible interpretations of the material which we have studied. These conferences will be followed by the writing of a report in which as many of the detailed conclusions as possible are included, both positive and negative findings are carefully evaluated, and next steps for further study clearly indicated.

6. Budget Plan:	Salaries*	3,450.
	Supplies, IBM equip.	800.
	& other overhead items	
*Investigator	2,250.	Total \$4,250.
Research Asst.	600.	
Statistical Asst.	600.	

(The above budget is estimated on the basis of about three-quarters of the Investigator's time during the three months of the project, and full time for the research assistant and statistical assistant during the middle period of the project. Miscellaneous expenses are estimated on our best guess as to the cost of supplies and equipment plus about 15% for overhead.)

7. Anticipated Duration of Work: Four months.

8. Facilities and Staff Available:

The Age Center of New England, Inc., a non-profit research facility, has an increasing pool of subjects which now consists of more than 700 mobile, apparently healthy men and women between the ages of 50 and 90. These subjects have declared and demonstrated their willingness to be studied in a variety of researches over a long period of time. The subject pool increases at the rate of more than 100 each year.

All research at The Age Center is under the direct supervision of an eight-man Research Council composed of senior men in the relevant disciplines, each of them outstanding in his field. The Research Council undertakes to be responsible for the quality of the research, the research personnel and the effective use of funds granted. Technically, it operates under responsibility delegated to it by The Center's Board of Trustees.

The Research Council consists of: Raymond D. Adams, M.D., Bullard Professor of Neuropathology, Harvard University; Hugh Cabot, M.B.A., Chairman of the Research Council, Trustee and Executive Director of The Age Center of New England, Inc.; Hudson Hoagland, Ph.D., Sc.D., Executive Director of the

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Worcester Foundation for Experimental Biology; Eugene M. Landis, M.D., Ph.D., George Higginson Professor of Physiology, Harvard University; Donald J. MacPherson, M.D., Psychiatrist and Trustee of The Age Center of New England, Inc; Harry C. Solomon, M.D., Professor of Psychology, Emeritus, Harvard University; Richard L. Solomon, Ph.D., Professor of Social Psychology, Harvard University; Samuel A. Stouffer, Ph.D., Professor of Sociology and Director of the Laboratory of Social Relations, Harvard University.

The Research Council, because of its close connection with many areas of research, have available to them consultants qualified to advise on any specific problem as it arises. These consultants at times work directly with the Research Council and at others with the principal investigators of the projects. These and similar staff will involve numerous staff conferences

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of supply):

Supplies. IBM equip. 500.

Administrative Assist. 500.

Signature

Frederick E. Whiskin, M.D., C.M.
Director of Project

Hugh Cabot

Executive Director of an

Research Council composed of senior faculty of the Harvard Medical School and the Massachusetts General Hospital. The Research Council is a non-profit organization and is not affiliated with any other organization. It is a separate entity and is not a part of the Harvard Medical School or the Massachusetts General Hospital. It is a separate entity and is not a part of the Harvard Medical School or the Massachusetts General Hospital.

Professor of Neurophysiology, Harvard University; Hugh Cabot, M.B.A., Chairman of the Research Council. Trustee and Executive Director of The Age Center of New England, Inc. and Executive Director of the Worcester Foundation for Experimental Biology.

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Curriculum Vitae

Frederick E. Whiskin,

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Born

REDACTED

A.B. University of Saskatchewan, 1944

M.D., C.M. McGill University, 1948

1948-50

1950-53

1953-55

1955-57

1957-

REDACTED

1958-

John Whiskin
Executive Director

1003537382

CONFIDENTIAL

TIRC Grant #241

Progress Report No. 1

Frederick E. Whiskin, M.D.
The Age Center of New England, Inc.

November 12, 1959

*project*Pilot Study of the Smoking Habits of Age Center Members

Dear Dr. Hockett:

This letter is to report to you informally on the "Smoking Study."

The response of our members to our smoking questionnaire was prompt. Over a 65% reply was received by early September. As I expected, Dr. Whiskin and certain of our other scientists became intrigued with the task and my problems have been to keep them within reasonable bounds.

The smoking data and the demographic, medical and psychological data in the form the scientists wished was sent some weeks ago to Littauer Statistical Laboratory at Harvard which we normally use for IBM calculations. There has occurred a predictable but unexpected delay in this data processing which should have been completed before now. Apparently we are trying to do too many things in too short a period of time and the programing became both complicated and expensive. I think the appropriate decisions have now been made and the correlations should be in our hands shortly. Some rearrangement of time assigned to this project has taken place and as soon as the analyzed data is completed we will be able to proceed expeditiously with its consideration.

In general, things seem to be under control but tentative conclusions will be reached somewhat later than I had hoped. Current conferences, however, indicate that the findings will also be richer.

Sincerely,

/s/ Hugh Cabot
Chairman

1003537383

Application for Research Grant

Date:

August 16, 1955

1. Name of Investigator:

William L. Williams, M. D.

2. Title:

Assistant Professor of Pathology (January 1, 1956)

3. Institution
& Address:

Institute of Pathology, University of Tennessee
838 Madison Avenue, Memphis, Tennessee

4. Project or Subject:

A proposed study of the effect of multiple respiratory infections
on the production of cancer of the lung.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

The experimental procedure may be divided into 4 phases as follows:

1) A survey of epithelial changes occurring in the bronchi of patients coming to autopsy in this Institution. The possible similarity in histogenesis between squamous cell carcinoma of the bronchus and squamous cell carcinoma of the endocervix should be noted. This theoretical similarity has been stressed by two recent case reports of intraepithelial carcinoma of the bronchus (Unker and Storey, Cancer 5:369, 1952 and Papanicolaou and Kuprowski, Cancer 4:141, 1951).

To study the incidence of occurrence of minor epithelial changes and intra-epithelial carcinoma the investigator will attend each autopsy and will remove the lungs and trachea in block. These organs will be fixed by the gentle injection of 10% formalin into the trachea. The first fluid injected will be aspirated and smears made which will be stained and studied in the usual manner for bronchial washings. After adequate fixation the lungs will be dissected by opening the bronchi including each branch of the segmental bronchi. Sections will be taken and labeled for each autopsy according to the segment (see enclosed diagram). This phase will answer the statistical question of the incidence of minor changes in the epithelium which may progress to cancer and will be correlated with phase #2. This same type dissection of lungs of patients with bronchogenic carcinoma may answer questions of the "field effect" of the carcinogenic stimulus.

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2) This phase is directed toward improvement of techniques for the earlier diagnosis of cancer of the lung thus lowering mortality and morbidity of this disease. It is in progress at present and consists of steps to improve the methods of collecting sputum from patients and an improvement in the method of preparing slides from the specimen. A blender is being utilized to break up mucous strands in sputum before the specimen is centrifuged. This will aid in concentrating any cells which may be in the sputum. The cells seen in the smears from living patients and those taken at autopsy may then be compared to the cells seen in the epithelium of the autopsy material. This will establish a better understanding of the significance of unusual cells seen in sputum and bronchial washings.

3) This is one of the most important phases and is directed toward learning the effect of multiple chronic irritation upon the development of cancer of the lung. It is to be recalled that Winternitz at Yale shortly after the great Influenza epidemic of 1918 pointed out and illustrated beautifully lesions in the lungs of people dying of influenza which now would be called intraepithelial carcinoma. Winternitz predicted at that time that cancer of the lung would be more frequent. Two animals will be used to study this question, the rabbit and swine. The rabbit will be used because of the lack of occurrence of clinical influenza in this animal. Multiple chronic irritation will be produced by injecting (as described by Dr. Sprunt in 1935) diphtheria toxin, staphylococcus toxin, and dilute acids into the trachea. After a suitable time lapse the lungs will be examined as described for the autopsy material.

The pig will be used because of the natural occurrence of influenza in this animal. It is probably that three different strains of virus may produce the disease in this animal (Dr. Hale - personal communication). The three infections may then, following waning of the immunity, be reproduced. This will then be followed by examination of the lungs for epithelial changes as described for the human and the rabbit. This phase should answer statistically the question of the effect of multiple infection and irritation upon the frequency of occurrence of epithelial changes and/or lung cancer. In order to study the reasons for the difference of occurrence of lung cancer between males and females each of the experimental groups will be divided into castrate and non-castrate animals. One additional group will be treated with the male sex hormone (testosterone).

4) This phase will utilize a new method of tissue culture which has been developed by the investigator to study the effects of the infection, irritants and hormones upon epithelial cells grown outside the influences of the body. Plastic chambers will be used to contain the cultures (see enclosed diagram). The pH of the media will be maintained at a constant level by a continuous gas flow through the chambers. The chambers will be sealed and submerged in a water bath to maintain constant temperature of the cultures and to allow instant observation of any gas leaks. Microscopic studies will be done to determine the effects of these agents on the epithelial cells after constant exposure.

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6. Budget Plan:

Salaries	\$ 29,400.00
Expendable Supplies	10,000.00
Permanent Equipment	11,050.00
Overhead	7,567.50
Other	500.00
Total	58,517.50

7. Anticipated Duration of Work: 5 years

8. Facilities and Staff Available: The most attractive factor is that of a large autopsy service handling approximately 900 autopsies per year. This will furnish adequate material for the studies of phase #1. CONSULTANTS: William M. Hale, Professor of Microbiology, Univ. of Tenn., at Memphis. Dr. Hale is the author of numerous papers in the field of immunology and has worked extensively with influenzal infections. Cyrus C. Erickson, Prof. of Pathology, Univ. of Tenn., Memphis, has written on the subject of Intraepithelial Carcinoma of Cervix and is immediately in charge of the Cancer of the Cervix Project and will be of excellent assistance in evaluating the changes found in the pulmonary epithelium. Douglas H. Sprunt, Professor of Pathology and Chief of the Division of Pathology and Microbiology, has worked extensively in the experimental production of changes in the pulmonary epithelium.

9. Additional Requirements:

The financial requirement will be reduced to approximately \$50,000 in subsequent years. This reduction will be primarily in permanent equipment.

10. Additional Information (Including: relation of work to other projects and other sources of supply):

Studies are at present underway in this institution concerning an apparently somewhat similar problem - squamous cell carcinoma of the endocervix. In studying the natural history of this disease, it is becoming apparent that many (though possible not all) squamous cell carcinomas of the endocervix go through a progression of squamous metaplasia, atypical metaplasia to intraepithelial carcinoma with ensuing invasion from these surface lesions. Studies to date from both human and animal material suggest that chronic inflammation is an important etiologic factor (Williams, William L., and Erickson, Cyrus C., Southern Medical Journal, submitted). This study underway includes the cytologic approach for population screening annually for a three year period. Statistical information gained in this manner should indicate time intervals or progression rates of these epithelial changes.

Signature s/ William L. Williams, M.D.
Director of Project

s/ Cecil O. Lipton
Business Officer of the Institution

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TIRC P. A.
Study
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Reprinted from the Proceedings of the NATIONAL ACADEMY OF SCIENCES
Vol. 45, No. 3, pp. 389-393. March, 1959.

SOME STATISTICAL OBSERVATIONS ON A COOPERATIVE STUDY OF HUMAN PULMONARY PATHOLOGY. II

By EDWIN B. WILSON AND MARY H. BURKE

OFFICE OF NAVAL RESEARCH, BOSTON, MASSACHUSETTS, AND TOBACCO INDUSTRY RESEARCH
COMMITTEE, NEW YORK, NEW YORK

Communicated December 26, 1958

In our first paper¹ we gave some general average data for the readings of eight pathologists in eight different cities on slides made from sections taken in standard positions in run-of-the-mill lungs at autopsy, using the following classifications: normal, hyperplasia, metaplasia, atypical metaplasia, carcinoma-in-situ and carcinoma. As carcinoma-in-situ was found so rarely by any of the pathologists, that classification will be combined with atypical metaplasia in this continuation of the study; there will be only five groups and their rank indices² will be 0, 1, 2, 3, 4.

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When we became convinced that the classification was being made on different bases by the different pathologists, we asked all twelve to read a selected sample of 40 slides. This they kindly did, and we reported on the considerable statistical differences in the readings. As the main object in all the work has been to obtain comparable data in the twelve cities for the degree of pathology in the lungs examined, we stated that it would be well to have a considerable sample of the slides from all cities read by several pathologists. The need for this is clear from the differences shown in Table 1 for the percentages of their slides placed in the 5 groups by the pathologists in eight of the twelve cities.³

TABLE 1

PERCENTAGE DISTRIBUTIONS FOR MALES, AGE 25 AND UP							
Reader	Slides	0	1	2	3	4	Index
J.....	909	28.8	53.6	11.7	4.2	1.8	0.97
D.....	941	57.1	21.1	7.7	11.1	3.0	0.82
A.....	408	38.7	46.1	15.0	0.0	0.2	0.77
E.....	630	66.7	9.7	18.7	3.6	1.3	0.63
B.....	223	65.9	9.4	21.1	2.6	0.9	0.63
L.....	2495	76.4	6.9	11.9	3.3	1.5	0.47
I.....	669	74.4	8.4	16.3	0.9	0.0	0.44
H.....	1418	81.8	9.7	8.0	0.4	0.1	0.27
Mean	...	61.2	20.6	13.8	3.3	1.1	0.62

We were fortunate enough to find three of the pathologists who were willing to read a sample of 609 slides drawn from the different cities by random processes.⁴ We included also the 40 slides previously read by all twelve. The present paper is a report on the results of the rereading. The two sets of slides will be treated separately. The gross results are in Tables 2 and 3.

TABLE 2

DISTRIBUTION OF TOTAL OF 609 SLIDES ON REREADING							
Reader	Slides	0	1	2	3	4	Index
A.....	609	359	93	127	14	16	0.744
E.....	609	348	25	212	6	18	0.885
L.....	609	357	88	133	7	22	0.760
Total	1827	1066	206	472	27	56	0.796

Reader A is high in atypicals (3) and Reader E is low in hyperplasia (1) and high in metaplasia (2) compared with the other two.

TABLE 3

DISTRIBUTION OF THE 40 SLIDES ON REREADING							
Reader	Slides	0	1	2	3	4	Index
A.....	40	4	4	27	3	2	1.875
E.....	40	5	2	28	2	3	1.900
L.....	40	5	6	25	1	3	1.775
Total	120	14	12	80	6	8	1.850

In this small sample, distributed very differently from the large one, the differences noticeable in the latter are not in evidence; but the distribution is significantly different from that previously found by all twelve pathologists, viz., 48, 120, 223, 57, 32; though it is not significantly different from what the three rereaders found, viz., 16, 20, 64, 10, 10.

The rereadings of the 40 slides by the three readers and their original readings

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different d sample statistical to obtain lungs ex- the slides from the in the 5

have the properties in Table 4. The first reader has not changed his mean significantly, the second has decreased his, and the third increased his, each significantly. The means thus have come closer together. The self-correlation coefficients vary from 0.65 to 0.86.

TABLE 4

Reader	Mean II	Mean I	Mean II - Mean I	Correlation $r_{I, II}$
A.....	1.875	1.800	+0.075 \pm 0.114	0.65
E.....	1.900	2.125	-0.225 \pm 0.103	0.76
L.....	1.775	1.525	+0.250 \pm 0.091	0.86

In the random sample, the numbers of slides belonging to A, E, and L, respectively, were 73, 72, and 60. The comparison of the rereadings by each of his own slides is given in Table 5. It is seen that the three pathologists are reading their own slides about as they did before and that the self-correlation coefficients⁵ are of about the same magnitude as for the 40 slides.

TABLE 5

Reader	Mean II	Mean I	Mean II - Mean I	Correlation $r_{I, II}$
A.....	0.548	0.644	-0.096 \pm 0.089	0.60
E.....	0.792	0.764	+0.028 \pm 0.073	0.81
L.....	1.150	1.333	-0.183 \pm 0.142	0.55

With this background we may turn to the standardization of the percentages over classes which result from using the rereadings of the three pathologists as a basis. The method is similar to that on standardizing death rates for age and sex against the age and sex distributions of a standard population. In Table 1, J put 28.8 per cent of his slides in the normals. The sample drawn for J from his 909 slides and presented to the three pathologists among other slides, contained 32 normals, 59 hyperplasias, 17 metaplasias, 5 atypicals, and 3 carcinomas. These were distributed by the three pathologists (averaged) as given in Table 6. We

TABLE 6

Rank	Number	0	1	2	3	4
0.....	32	31	$\frac{2}{3}$	$\frac{1}{3}$	0	0
1.....	59	$38\frac{1}{3}$	13	$7\frac{1}{3}$	$\frac{1}{3}$	0
2.....	17	3	$\frac{2}{3}$	$10\frac{2}{3}$	$2\frac{2}{3}$	0
3.....	5	2	$\frac{1}{3}$	$1\frac{2}{3}$	0	1
4.....	3	0	0	$\frac{2}{3}$	$\frac{1}{3}$	2

have to assume that all J's slides of each class would have been distributed in these same proportions had they all been reread. Thus his 28.8 per cent of normals in Table 1 would have been distributed as $\frac{31}{32}$ of 28.8 per cent normals, $\frac{1}{48}$ of 28.8 per cent hyperplasia, and $\frac{1}{96}$ of 28.8 per cent metaplasia. In this way one calculates Table 7.

TABLE 7

Original	0 28.8	1 53.6	2 11.7	3 4.2	4 1.8
0.....	27.9	0.6	0.3	0.0	0.0
1.....	34.8	11.8	6.7	0.3	0.0
2.....	2.1	0.4	7.3	1.8	0.0
3.....	1.7	0.3	1.4	0.0	0.8
4.....	0.0	0.0	0.4	0.2	1.2
Standardized	66.6	13.1	16.1	2.3	2.0

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The comparison of J's original percentages at the top of this table with the adjustment by the averaged readings of the three pathologists reveals the fact that they would have read his slides very differently and would indeed have given for them a percentage distribution not very far from the mean. This does not mean that J was wrong and they are right; it only means that there is a difference. Treating all eight in the same way, Table 1 as adjusted becomes Table 8.

TABLE 8

ADJUSTED PERCENTAGE DISTRIBUTIONS FOR MALES, AGE 25 AND UP							
Reader	Slides	0	1	2	3	4	Index
J.....	909	66.6	13.1	16.1	2.3	2.0	0.60
D.....	941	62.7	13.3	18.0	0.8	5.1	0.72
A.....	408	63.9	15.3	19.9	0.0	0.9	0.59
E.....	630	68.5	6.5	22.5	1.8	0.8	0.58
B.....	223	74.3	7.6	14.0	1.0	3.0	0.51
L.....	2495	60.9	12.2	21.0	0.9	5.0	0.78
I.....	669	71.1	5.2	20.3	0.5	3.0	0.58
H.....	1418	68.8	13.3	13.6	0.6	3.7	0.57
Mean.....	67.1	10.8	18.2	1.0	2.9	0.62

When one compares Tables 1 and 8, bearing in mind that, had any three other pathologists reread the slides, the adjustments would have been different,⁶ and further bearing in mind that the adjustments have been made by scaling up samples in the different cities of from 60 to 75 with one exceptionally large one of 116, it is obvious that most of the differences between the eight cities have disappeared and that it would be very difficult to separate out from the adjusted percentages items which proved that the pathological conditions of the lungs in the different cities were in fact different.⁷

Even when comparisons of general morbidity or mortality conditions in different places are dubious because of differences in reporting, the analysis of local reports by those familiar with local conditions has value. We hope that the individual pathologists who have been good enough to engage in this survey will work up their data in any way they please. We shall be glad if our study furnishes them something of value for theirs.

¹These PROCEEDINGS, 43, 1073-1078, 1957.

²This will make the mean indices, standard deviations, and correlation coefficients of the previous paper not strictly comparable with those here, but the comparison will not have to be made.

³It has been necessary to omit four of the twelve cities. One of the co-operating pathologists failed to send in the data from his city; one had so few cases that it seemed better not to include his city in the rereading; one sent in no slides to be reread; one had used the Swiss roll instead of the standard sections, and we feared this might introduce noncomparability.

⁴The 609 slides are not strictly random because a few more had been drawn randomly, of which some had to be discarded because at least two of the three rereaders felt that they were not good enough to be read at all. It is, however, our belief that this loss did not seriously disturb the randomness.

⁵The self-correlation coefficients have long been used by psychometrists, educational testers, and others to give one estimate of the reproducibility of the data. See, for example, C. Spearman, *The Abilities of Man, Their Nature and Measurement*; T. L. Kelley, *Crossroads in the Mind of Man: A Study of Differentiable Mental Abilities*; J. P. Guilford, *Psychometric Methods*. On pages 411 ff. of the last is given a brief general discussion of various concepts related to reliability. Our index is a rank index, an index of ordinal position. So are many, if not most, of the grades or marks which teachers use. It may be questionable whether one should treat ranks as cardinal numbers, but that is widely done as we are doing it.

The mean value of the three self correlations on the forty slides is 0.76 ± 0.06 , and of those on their own slides is 0.65 ± 0.09 . We have six mutual correlations of the three pathologists in pairs on the forty slides and six on their own; the values of the means are respectively 0.69 ± 0.03 and 0.58 ± 0.04 . Owing to the small numbers in the samples these means have little statistical stability; but so far as the evidence goes, it indicates that the self correlations are not much larger than the mutual correlations. Or in other words, the three pathologists reproduce one another's readings about as well as they reproduce their own—as measured by these correlations. The natural interpretation is that their remaining differences are chiefly fortuitous or random, due to lack of definition and possibly to lack of complete definability of the pathological material. Some slides may be far from clear; should they be discarded? Some may have part of the mucosae lacking; what about them?

If n, h, m, a, c be the fractions (probabilities) of slides of a certain area on which the worst condition is normal, hyperplasia, metaplasia, atypical metaplasia, and carcinoma, what would be the fractions on slides which had twice that area? This question cannot be answered with any information we have; but it is interesting to consider and may suggest interesting research. If the condition revealed by the slide were so widespread that it would occur on both halves of the slide of double area, there would be no differences in the probabilities. At the other extreme where the (worst) condition is so sharply localised that the condition on the slide had no relation to that on an adjacent equal area, the fractions for slides covering a doubled area could be obtained from combinations of terms in the expansion of $(n + h + m + a + c)^2$. As an illustration, if for slides covering a given area, the fractions (probabilities) are $n = .70, h = .10, m = .15, a = .03, c = .02$, then the results for the slides covering twice the area would be 0.49, 0.15, 0.26, 0.06, 0.04, respectively. If the work were to be done over, it might be well to record enough about the conditions appearing on the slides to learn something about their correlations. Such a study might reveal evidence bearing on the question whether in truth the five conditions are in fact successive.

* The two cities, J and H, top and bottom of Table 1, which showed the highest and the lowest values of the index and the lowest and highest percentages of normals, were each first adjusted by using the rereadings of each of the three pathologists, and the results were in fact different, as must be expected; but a study of their similarities indicated that an averaging of the findings of the three pathologists should give not only a stabler result but one which would give a standardization worth having.

† Consider, for example, what the rereading by A, E, L has done to their own previous distributions:

	A Old	A New	E Old	E New	L Old	L New
Index.....	0.77	0.59	0.63	0.58	0.47	0.78
Per cent normal.....	38.7	63.9	66.7	68.5	76.4	60.9

For these three the mean index was 0.62 and has become 0.65—an insignificant change. The differences from the old mean index were +0.15, +0.01, -0.15; from the new -0.06, -0.07, +0.13. Descriptively the correlation is negative, though not significant. The differences from the respective means of per cent normal were -21.9, +6.1, +15.8 and become -0.5, +4.1, -3.5, and again the correlation is negative. This is simply an indication of the differences inherent in passing judgments on the slides. If we correlated the two sets of per cent normal in Tables 1 and 8, we would find $r = 0.24$, and if we correlated the two sets of indices, $r = 0.05$. The striking phenomenon to notice is how much the standardization has reduced scatter.

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TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

Application For Research Grant

Date: November 3, 1954

1. Name of Investigator: Robert C. Wilson and Leslie H. Squier (co-investigators)

2. Title: Associate Professor and Instructor, Psychology Dept.

3. Institution & Address: Reed College, Portland 2, Oregon

4. Project or Subject: A study of the relationships between personality patterns, smoking behaviors, and lung cancer, cancer, and heart diseases.

It is hypothesized that the major relationship between smoking and the occurrence of lung cancer, cancer, and heart disease is not a causal one. Rather, it is hypothesized that both smoking and the occurrence of these diseases are in part dependent upon antecedent personality factors. Parenthetically, these personality factors may be related to antecedent physiological or biochemical factors which are beyond the scope of the present investigation.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

The complete study is conceived of in four phases:

Phase 1: An empirical study of personality differences between cigaret smokers, pipe smokers, cigar smokers, and non-smokers, using the Minnesota Multiphasic Personality Inventory, a self-description adjective check list, and a specially constructed questionnaire concerning a variety of specific smoking behaviors hypothesized to bear a relationship to personality differences. This phase, to be carried out on readily available groups, would yield:

- 1) A scale of objective personality items differentiating various types of smokers from non-smokers and from each other.
- 2) A determination of significant differences in self-description and specific smoking habits.

In addition, this phase will serve to delineate more sharply the hypotheses to be tested in Phase 2.

Phase 2: Depth interviews of a stratified sample of adult cigaret, pipe, and cigar smokers and non-smokers. The interview technique provides an opportunity for a fuller exploration of some of the hypothesized differences in personality pattern than is possible by the methods employed in Phase 1. In addition, this phase will provide a cross-validation of the findings of Phase 1.

Phase 3: An extension of the study to lung cancer, cancer, and heart disease patients, using the techniques and findings developed in Phases 1 and 2. Personality comparisons will be made between smokers with cancer, non-smokers with cancer, and smokers and non-smokers without cancer. It should be possible to develop a scale of cancer-proneness based on personality items, which would be more predictive of cancer than would smoking or non-smoking behavior.

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Subjective somewhat new
poss. may not be
definitive
Phase 3 rough

good approach

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Phase 4: A follow-up study of the subjects used in Phase 2, or a study of individuals being admitted to a hospital for various ailments, including cancer. The Cancer-Proneness scale could be validated by administration prior to diagnosis.

The present proposal requests funds to cover Phases 1 and 2 only, since they will require approximately a year to complete. Upon completion of these phases, a detailed proposal will be submitted for Phases 3 and 4.

Phase 1, Supplementary Information

First there will be a more detailed survey of the literature, both for the purpose of coordinating the outlined research with previously-conducted studies, and to provide additional hypotheses.

The subjects used will be several hundred students at various colleges and universities, and employees of certain state institutions where the MMPI, ~~Minnesota Multiphasic Personality Inventory~~ (Minnesota Multiphasic Personality Inventory) is routinely administered. This will provide a large sample representing more than one geographical area and socio-economic level, at a maximum economy of time and expense. The MMPI, developed initially as a clinical test, has proved useful in differentiating groups according to various non-clinical behavioral criteria. Personality Scales can be developed to differentiate various types of smokers and non-smokers, and give additional information as to the personality patterns characteristic of these types.

The self-descriptive adjective check list is compiled of common adjectives used to describe people in every-day speech. Each subject checks those adjectives which he believes to describe himself as he really is. This procedure, widely employed for personality assessment, would provide a clear picture of differences between self-descriptions of the various smoker and non-smoker types, to supplement the descriptive material from the MMPI.

The questionnaire of specific smoking habits will be based upon a preliminary survey of such habits believed by smokers themselves to be of relative importance, such as the quantity of cigarettes smoked per day, inhaling, smoking before breakfast or during periods of interrupted sleep, etc. From the data provided by this questionnaire, it will be possible to establish empirically various categories of smokers and non-smokers, as well as to make a preliminary outline for the interview schedule to be used in Phase 2.

IBM machine methods will be used rather extensively in analyzing the data derived from this phase. Use of these methods enables a considerable economy in expenses for clerical operations.

At the completion of Phase 1, a report will be prepared presenting the findings up to that point.

Phase 2, Supplementary Information

Depth interviews will be carried out during the middle of 1955, so as to make use of the analyses of the data from Phase 1. Approximately 600 interviews will be conducted using a stratified sample of smokers and non-smokers. This phase will not only provide validation of the material from Phase 1, but will supplement this with information about the motivational patterns which differentiate the smoker from the non-smoker. As is apparent in the attached budget, the expense of obtaining and analyzing interview data is much greater than that involved in Phase 1. However, the interview enables the use of projective methods designed to reveal aspects of personality which are not readily accessible through the use of paper-and-pencil tests.

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This sort of procedure requires well-trained, intelligent interviewers. A number of such people are available in this area for employment during the summer months. Again, IBM methods will be used to carry out routine clerical operations insofar as possible.

A comprehensive progress report will be prepared at the completion of Phase 2, presenting the findings of both phases. Such interim reports would be prepared as seemed desirable to the Committee.

DETAIL OF BUDGET PLAN

Phase 1

Professional Salaries	\$4,590.00	
Clerical Assistance	1,530.00	
Tests & Questionnaires	1,000.00	
Calculator	750.00	
IBM Estimated Costs	2,500.00	
Travel	<u>1,000.00</u>	
	11,370.00	
Overhead at 20%	<u>2,274.00</u>	
Total, Phase 1		13,644.00

Phase 2

Professional Salaries	6,120.00	
Interviewers' Salaries	6,120.00	
Analysis and Coding of Interviews	6,120.00	
Clerical Assistance	1,530.00	
IBM Estimated Costs	2,500.00	
Travel	<u>1,000.00</u>	
	23,390.00	
Overhead at 20%	<u>4,678.00</u>	
Total, Phase 2		26,010.00

Overall Total, Phases 1 & 2

\$41,712.00

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6. Budget Plan:

Phases 1 and 2
(see detailing on other
page)

Salaries	\$25,970.00
Expendable Supplies	1,000.00
Permanent Equipment	750.00
Overhead	6,952.00
Other	7,000.00
Total	\$41,712.00

7. Anticipated Duration of Work:

Phase 1: 4 to 6 months

Phase 2: 6 to 8 months

8. Facilities and Staff Available:

All necessary facilities and staff are available.

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of supply):

Signature Leslie H. Squier
Director of Project

W. C. Taylor
Business Officer of the Institution

Controller

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Application For Research Grant

*See HRP 108
sounds good re: cheap
source of components -
which ones*

Date: July 28, 1955

1. Name of Investigator: **Travis Winsor, M.D.**

2. Title: **Assistant Clinical Professor of Medicine, University of Southern California, Chairman, Board of Directors, Heart Research Foundation, Director, Nash Cardiovascular Research Foundation and Heart Department, Hosp. of the Good Samaritan.**

3. Institution

3875
& Address: **Heart Research Foundation, 3875 Wilshire Boulevard, Los Angeles 5, Cal. and The Hospital of the Good Samaritan, 1212 Shatto Street, Los Angeles 17, Cal.**

4. Project or Subject: **Effect of tobacco and/or its constituents upon the vascular system of man in health and in various disease states.!**

5. Detailed Plan of Procedure (Use reverse side if additional space is needed): **It is proposed to make measurements of the peripheral and central (heart) circulation before and after the administration of tobacco and/or its component parts. The following would be studied:**

1. **Digital circulation by means of measurements of volume pulsations using an electronic pneumoplethysmograph, blood flow employing a venous occlusion technique and the plethysmograph, vascular volume measured after intra-venous injection of radioactive iodinated albumin 131 employing a recording gamma ray spectrometer and skin temperatures using recording thermocouples or thermistors.**
2. **Calf muscle circulation by means of measurements of skin temperatures, muscle temperatures and clearance rate of radioactive sodium.**
3. **Kidney circulation by determining renal plasma flow using para aminohippurate.**
4. **Blood pressure, pulse rate and cardiac output using for the latter the dilution of radioactive agents.**
5. **Oxygen consumption to determine the effect on body metabolism.**

It is proposed to measure these ~~smoke~~ modalities before and after smoking corn silk (Cubeks), standard brands, "de-nicotinized" brands, and various filtered brands of cigarettes. We are especially interested in studying the effects of chemical agents derived from tobacco other than nicotine to determine if substances favoring vasodilation can be detected.

X **As tobacco smoking produces strong negative ionization of oxygen we would intend to study the effect of ionized oxygen using radioactive polonium to determine if "negative oxygen" is in itself a cause of the vasoconstriction reported from tobacco smoking.**

Enclosed are reprints which describe some of the studies we have made employing methods described above.

1003537399

6. Budget Plan:

Salaries	Part-time technician	\$1,000.00
Expendable Supplies		400.00
Permanent Equipment	Gamma ray spectrometer	5,500.00
Overhead		None
Other		None
Total		\$6,900.00

7. Anticipated Duration of Work:

One to two years.

8. Facilities and Staff Available:

Staff of the Heart Research Foundation: one Fellow (M.D.), half-time; one technician, half-time; one secretary, half-time; one electronic engineer, half-time; and one photographer, part-time. Facilities and staff of the Nash Foundation also will be employed. This includes one secretary and two technicians and the use of the following equipment: Two plethysmographs with recording oscillometers, two multipoint Brown potentiometers, a temperature-controlled room with controlled humidity and air flow, oxygen ionizers and thermister thermometers.

9. Additional Requirements:

We will require chemical agents isolated from tobacco and tobacco prepared by various methods for study. We would need nicotine in its ~~an~~ chemical state. It would be our intention to obtain these agents through chemists associated with your organization.

10. Additional Information (Including relation of work to other projects and other sources of supply):

This work would be carried on in conjunction with studies currently being carried out on vasomotion and factors regulating blood flow to the periphery.

We would greatly appreciate your consideration of this project as we are planning our program for the coming year.

Sincerely,

Signature

Dr. Travis Winsor, M.D.

Wiley Winsor

Business Office of the Institution

1003537400

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

Application For Research Grant

Date: July 29, 1954

1. Name of Investigator: **CHARLES ARTHUR WOERNER Ph.D., M.D.**
2. Title: **ASSOCIATE PROFESSOR OF ANATOMY**
3. Institution & Address: **SCHOOL OF MEDICINE, UNIVERSITY OF LOUISVILLE
101 WEST CHESTNUT STREET, LOUISVILLE 2, KENTUCKY**
4. Project or Subject: **A STUDY OF THE EFFECT OF TOBACCO TARS AND EXTRACTS ON THE
ARTERIES OF EXPERIMENTAL ANIMALS**

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Tobacco tars, and selected tobacco extracts will be injected into experimental animals. In the first series these materials will be injected directly into the walls of the arteries to be studied, and after intervals of 12, 24, and 48 hours, then 4, 8, and 16 days then the arteries will be fixed in suitable solutions and the sections stained for the demonstration of fats or of the connective tissues most often involved in arteriosclerosis. In a second series the tobacco materials being studied will be added to emulsions of fatty materials and injected intravenously. Histological, and histochemical examination of the arteries will then be made to demonstrate any degenerative changes that may occur. Comparison will be made between the effects of the tobacco materials and other chemicals.

1003537401

6. Budget Plan:

Salaries	\$5,200
Expendable Supplies	\$2,466
Permanent Equipment	\$1,500
Overhead	\$334
Other	
Total	\$9,900

7. Anticipated Duration of Work:

Three years, it is estimated that the budget of the second year would be \$8,900, and \$8,400 the third year.

8. Facilities and Staff Available:

Dr. C. A. Woerner would direct this research, Dr. Richard Taylor of the Dept. of Pathology would collaborate on the interpretation of the tissue sections.

Four students have been trained in the animal surgery and histological and histochemical techniques. Three of these will be available for work on this project the fourth will receive the M.D. degree before this project can be started. Dr. Noer and Dr. Kleinert of the Dept. of Surgery will advise on the techniques of injecting the arteries. No difficulty has been encountered in the current series of injection experiments.

9. Additional Requirements:

The permanent equipment needed includes an air conditioning unit (\$450), to maintain the laboratory temperature in a range that will make the histochemical studies possible, in June, July, and August the temperature here in Louisville is too high for these procedures to be carried out satisfactorily. A Refrigerator (\$300) is required to keep perishable materials, and specimens. A photomicrographic apparatus (\$750) is required to prepare illustrations of quality for clear publication of results.

10. Additional Information (including relation of work to other projects and other sources of supply):

A project entitled A STUDY OF LIPIDS AND MUROPOLYSACCHARIDES IN FAILING ARTERIES is being conducted under a grant of \$9,160 from the National Heart Institute.

The proposed project will be a continuation of this series of studies on the factors significant in the etiology of arteriosclerosis and other arteriopathies.

Signature _____
Charles A. Woerner Ph.D. M.D.

Business Officer of the Institution

1003537402

36
TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

*Indication
Prob. valuable
Assumed missing OK*

Application For Research Grant

Date: October 8, 1954

1. Name of Investigator: *A* William W. Wolff, A.M., Ph.D.

2. Title: Associate Professor of Clinical Chemistry and Toxicology

3. Institution & Address: Bowman Gray School of Medicine
South Hawthorne Road, Winston-Salem, N. C.

4. Project or Subject: A: The Fate of Tars from Cigarette Smoke Deposited in the Dog Lung
B: Cigarette Smoke in the Human Lung, a Radioisotope Study

5. Detailed Plan of Procedure (Use reverse side if additional space is needed)

A: By means of carbon-14 as an isotope tracer, an extensive experimental study will be made on the deposition and removal of tar components of cigarette smoke deposited in the dog lung. This will be a continuation of studies now being completed. (See statement below.)

A study is planned in three phases. The first phase will be the development of an economical and efficient method for introducing a carbon-14 label into tar constituents of cigarette smoke. Following the technique used in our studies with other isotopes, various types of organic compounds labeled with carbon-14 will be introduced into the cigarette just prior to smoking. The smoke from the treated cigarette will be condensed in a flask cooled with dry ice and the carbon-14 activity of the tar will be measured. Various procedures will be used to determine the nature of the carbon-14 linkage in the tar. As a second method, compounds such as glucose or amino acids containing carbon-14 will be introduced as a nutrient solution into the green tobacco leaf which will then be processed into cigarettes. The latter method should give a "true label" by actually incorporating carbon-14 atoms into normal constituents of the tobacco. This part of the study might throw light on the mechanism of tar formation.

The second phase of the study will be acute smoking experiments with dogs. The pattern of tar deposition in the lung will be determined. Removal of tar from the lungs will be studied as to (a) rate in each area (alveoli versus large bronchi, etc.,) (b) pathway of removal and (c) distribution in other body tissues. The metabolism of carbon-14 labeled tars introduced into the lung will be followed in considerable detail.

The third phase of the study will be long term smoking experiments extending over weeks or months. By using a recently reported technique for introducing a plastic tracheal cannula which remains in place for months, it will be possible to give a dog several doses of smoke for an extended period of time. This program will, to a considerable degree, simulate the pattern of smoking in the human. A study of the metabolism of cigarette smoke tar in the dog under these conditions should suggest the course of events in the human smoker.

1003537403

5 continued --

B: The deposition and fate of cigarette smoke in the human lung will be studied by means of radioactive iodine - 131 as a tracer in smoke. Expected results should give new and unique information on (a) the pattern of deposition of smoke particles in the bronchial tree, and (b) the speed at which the smoke deposit leaves the lung.

The feasibility of such experiments has been demonstrated by extensive observations on the dog. The amount of isotope appearing in the smoke, in any dose between 1 and 100 microcuries, can be fixed by the amount of radioactive iodine - 131 as potassium iodide added to the cigarette prior to smoking. Over 90% of the activity (smoke particles?) disappears from the dog lung within a few seconds after the smoke is deposited. The remainder of the radioactive material leaves the lung over a period of 30 to 60 minutes. A somewhat similar result may be expected in the human.

Subjects in this study will be patients undergoing a test of thyroid function under the care of J. Robert Andrews, M.D., Director of the Department of Radiology, N. C. Baptist Hospital. The subject will inhale cigarette smoke containing 50 to 100 microcuries iodine-131. The deposition of radioactive material in the lung and its rapid phase of removal will be measured by a scintillation counter and a high speed scaler on which readings will be made at one or two second intervals during the smoking period by photographing the scaler dials and interpolation lights. At the end of the smoking period the pattern of smoke deposit, the residual radioactivity in the lungs, will be mapped by scanning the entire lung field with the counter collimated to record only a 3 to 5 cm² area at each reading. (Chest plates will be obtained for comparison.) Repeated readings over a single area for an hour or more will indicate the speed at which the residual deposit leaves the lungs.

Application for authorization to make this study is being filed with the Atomic Energy Commission by Dr. J. Robert Andrews as clinician-in-charge.

1003537404

Enclosed with Dr. Wolff's application were the following, which are in our Scientific Files:

1. "The Effect of Tobacco on Estrus, Pregnancy, Fetal Growth, and Lactation" by Rowland V. Long, M.D., Lexington, N.D.; and William A. Wolff, Ph.D., Winston-Salem, N. C.
2. "Radioautographic method for studying deposition of cigarette smoke in the dog lung" by William A. Wolff, James G. Tuttle and John M. Godfrey
3. "The Use of Radioisotopes as Tracers in Cigarette Smoke" by William A. Wolff, Ph.D., E. G. Purdom, Ph.D. and J. A. Isenhower, B.A.
4. "Studies on tobacco chewing" by William A. Wolff and W. E. Giles
5. "The Spectrophotometric Estimation of Nicotine in Blood" by William A. Wolff, Marina A. Hawkins and W. E. Giles
6. "Nicotine in Blood in Relation to Smoking" by William A. Wolff, Marina A. Hawkins and W. E. Giles

1003537405

6. Budget Plan: one year, from 1-1-55 thru 12-31-55

request consideration of grant
as initial support on long term
program.

Salaries
Expendable Supplies incl. C-14 cpds.
Permanent Equipment
Overhead
Other alterations

\$13,000.00
6,000.00
2,000.00
2,000.00
2,000.00
Total \$25,000.00

7. Anticipated Duration of Work:

Project A - 3 to 5 years. Project B - 18 to 24 months.

8. Facilities and Staff Available:

Isotope laboratory, A.E.C. approved, 900 ~~XXXX~~ square feet, 5 rooms, 2 hoods, air conditioned

Chemical laboratory, 550 sq. ft., 2 rooms used for tobacco research only.

Staff: William A. Wolff, Biochemistry (part time)

E. G. Purdon, Ph.D. in physics, electronics and instruments (part time)

J. Robert Andrews, M.D., Clinician-in-charge of human subjects, Project B

Two technicians, chemical (full time)

X-ray technician, photographer and resident in radiology (part time as needed)

9. Additional Requirements:

- (1) Heavy lead shield for scintillation counter
- (2) Time interval movie camera for high speed recording on scaler
- (3) Rebuild one room in animal quarters to accommodate dogs receiving carbon-14 compounds over long periods of time.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The projects proposed above are a direct continuation of a research program which was started in 1944 and has been subsidized in its entirety by the R. J. Reynolds Tobacco Company through its agent, Wm. Esty, Inc. of New York City. During the early years of the program attention was directed to the development of suitable micro methods for a study of nicotine metabolism in the human smoker subject. Investigations on methods and blood nicotine levels in relation to smoking have been published. A study of nicotine absorption from chewing tobacco and a study on the ~~xxx~~ nicotine content of breast milk have been reported but not yet published.

Since April 1951 all effort has been concentrated on a study of isotopes as tracers in cigarette smoke. One paper has been published, two others have been reported, and a third is being completed at the present time. Results obtained to date show that, with a smoke dose and a smoking rate similar to that used by the human smoker, 90 to 98% of the nicotine in the smoke deposit disappears from the dog lung during the period of smoking and the remainder leaves the lungs during the next 30 to 60 minutes. Radioactive sodium or potassium introduced with the smoke leaves the dog lung almost instantly while radioactive iodine and arsenic are removed in about the same manner as is nicotine.

These ~~experiments~~ experiments with radio isotopes have been made on dogs with the object of getting fundamental information on the physiology of smoking.

Signature /s/ William A. Wolff
Director of Project

/s/ Harry O. Parar
Business Officer of the Institution

1003537406

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

Renewal

97 R1

Application For Research Grant

Date: March 28, 1956

1. Name of Investigator: J. Edwin Wood

2. Title: Instructor in Medicine,
Boston University School of Medicine

3. Institution
& Address: Massachusetts Memorial Hospitals
Evans Memorial
65 East Newton Street
Boston 18, Massachusetts

4. Project or Subject:

The acute effect of inhalation of tobacco smoke upon
reactive hyperemia blood flow of the foot in normal
individuals and patients with peripheral vascular disease.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed): The purpose of this study would be to determine whether or not the vasoconstrictor response of the foot blood vessels to tobacco smoking impairs the response of these blood vessels to vasodilator ischemic stimulus. Studies in our laboratory of the resting circulation of the foot indicate that the vasoconstriction induced by smoking is of little consequence even if the subject smokes after prolonged abstinence or if he smokes in a cool environment. The question remains as to whether or not this vasoconstrictor stimulus, however mild, impedes the maximal blood flow response to the severe stress of local ischemia. If tobacco smoking does not impair the response to this stress than further evidence favoring the general thesis that peripheral vasoconstrictor effects are inconsequential would be added. Subjects to be tested would consist primarily of patients with occlusive vascular disease since this group uniformly exhibits an impaired reactive hyperemia response following local arterial occlusion. Further impairment or the absence of further impairment of this response due to smoking would be more significant in this group of patients. Normal professional subjects will also be tested in the same manner for further comparison of data.

The test is to be carried out as follows: The subject will be exposed to a room temperature of 83° F for 30 minutes or whatever time after that required for stabilization of basal blood flow and skin temperature readings for a control 15 minute period. Then he will begin smoking two cigarettes at his own rate and depth of inhalation. After five minutes arterial occlusion will be applied to the foot for 5 minutes then reactive hyperemia blood flow will be measured. Reactive hyperemia blood flow without smoking will also be measured. The method of measurement and analysis of results will be the same as outlined by Wood, Litter and Wilkins, Circulating Research 3: 581, 1955, KMMX. Analysis of results will allow comparison of maximum post-ischemia blood flow rate and total post-ischemia blood flow in excess of prior resting flow with and without smoking.

1003537407

6. Budget Plan: Salaries for technical and secretarial assistance will be \$3,150 while payment to normal professional subjects will require \$150.

Salaries	\$3,300.00
Expendable Supplies	300.00
Permanent Equipment	- - - -
Overhead	400.00
Other	- - - -
Total	\$4,000.00

7. Anticipated Duration of Work:
One year

8. Facilities and Staff Available: Facilities are essentially the same as outlined in our previous proposal.
The project is to be directed by Dr. J. Edwin Wood. Dr. John W. Eckstein will not be with us in the coming year.
Miss Barbara Sears, who has participated in all of the experiments carried out during the present project year, has gained enough experience to take over many tasks performed by Dr. Eckstein. Thus no difficulties are anticipated from this point of view. Dr. Robert W. Wilkins, Director of the Cardiovascular Division of the Evans Memorial Hospital will be available at all times for consultation.

9. Additional Requirements:

None.

10. Additional Information (Including relation of work to other projects and other sources of supply):

This project, we believe, is a natural outgrowth of the present study of tobacco and the peripheral vascular system being carried out in these laboratories. A detailed report of studies to date was given at a recent meeting with T.I.R.C. representatives in New York.

Signature /s/ J. Edwin Wood
Director of Project

/s/ Philip D. Bennet M.D.
Business Officer of the Institution

Administrator

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

97

Application For Research Grant

Date: May 31, 1955

Name of Investigator: J. Edwin Wood, M.D.

2. Title: Instructor in Medicine, Boston University School of Medicine

3. Institution
& Address: Massachusetts Memorial Hospitals
Evans Memorial
65 East Newton Street
Boston 18, Massachusetts

4. Project or Subject: The effect of prolonged inhalation of tobacco smoke and of prolonged abstinence from the use of tobacco on the peripheral vascular response to acute inhalation of tobacco smoke in man.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed): The purpose of this study would be to determine whether or not the acute response of the peripheral vascular system to the inhalation of tobacco smoke is influenced by the presence or absence of habitual smoking prior to the test. Two groups of subjects will be used; one, normal young individuals with no vascular disease, and two, patients suffering from various forms of peripheral vascular disease.

Subjects will be tested three times during their normal smoking habit, following 24 hours of abstinence, and following 7 days of abstinence.

The test is to be carried out as follows: The subject will be exposed to a room temperature of 83°F. for 30 minutes or whatever period of time that is required for the achievement of basal blood flow and skin temperature readings of the feet for a 15 minute period. Then he will smoke one cigarette during which and after which blood flow and skin temperature determinations will be repeated until the values return to control levels. The entire procedure will be repeated with the subject exposed to a room temperature of 68°F.

These two room temperatures are used by us in standard testing in order to evaluate vascular response under conditions of mild vasodilatation (warm room) and mild vasoconstriction (cool room).

Blood flow in the foot will be measured by the standard venous occlusion method with a full foot plethysmograph. Blood flow change will be estimated by the change in pulse volume of the great toe of the opposite foot as measured by a digit plethysmograph. Skin blood flow of a small toe will be estimated by continuous recording of skin temperature from an attached thermocouple.

1003537409

6. Budget Plan:

Salaries for technical and secretarial assistance will be \$3,000, while payment to ten normal professional subjects for three tests each will require \$300.

Salaries	\$3,300.00
Expendable Supplies	300.00
Permanent Equipment	0.00
Overhead	400.00
Other	0.00
Total	\$4,000.00

7. Anticipated Duration of Work:

One Year.

not much money

8. Facilities and Staff Available: Facilities consist of two adjacent peripheral vascular testing laboratories, one of which is a constant temperature room. All necessary plethysmographic and skin temperature measuring equipment is present in the laboratories. General medical and general surgical wards are present in the same building and are a ready source of hospitalized patients with peripheral vascular diseases. The peripheral vascular Out Patient Department, which is attended by the proposed director of the project, is also a source of patient material. Normal subjects will be volunteer medical students.

Staff to consist of the Director of the Project, ~~and Dr. Robert M. Wilkins, Director of the Cardiovascular Laboratories of the Evans Memorial Hospital, who will devote full time to the project and Dr. John W. Eckstein, who will devote full time to the project and Dr. Robert M. Wilkins, Director of the Cardiovascular Laboratories of the Evans Memorial Hospital, who will be available for consultation.~~

necessary technical & secretarial assistance will be available.

9. Additional Requirements: There are no additional requirements.

10. Additional Information (Including relation of work to other projects and other sources of supply):

Studies have been carried out in the past which suggest that vasoconstriction occurs in response to tobacco inhalation. The question of possible modification of this response by prior experience with tobacco in normal and diseased patients has not been carefully studied. Though the possibility that "cutting down" on tobacco may actually evoke a more profound response when it is occasionally used than with continued use has been suggested. Such has never been systematically studied to the knowledge of the undersigned however.

Signature /s/ J. Edwin Wood
Director of Project

/s/ Philip D. Bennet, M.D.
Business Officer of the Institution Administrator

1003537410

CONFIDENTIAL

TIRC Grant #97

Progress Report #1

Dr. J. Edwin Wood
Massachusetts Memorial Hospitals
Evans Memorial

January 4, 1956

"The Effect of Prolonged Inhalation of Tobacco Smoke and of Prolonged
Abstinence from the Use of Tobacco on the Peripheral Vascular
Response to Acute Inhalation of Tobacco Smoke in Man"

Dr. John W. Eckstein, who is spending the year with us from the State University of Iowa College of Medicine, and I have completed most of the contemplated work on normal subjects. Ten subjects have been studied forty times in warm and cool environments. Fourteen of these were paired studies with and without abstinence from smoking prior to the experiment.

Results so far indicate that, under conditions of the experiment, prior abstinence from smoking does not alter the response to smoking. In addition, it has been noted that the vasoconstriction induced by the cool environment is greater than that produced by smoking alone.

A scientific report of the results of this study will be given at the February 13 meeting of the New England Cardiovascular Society.

The remainder of the year will be devoted to carrying out these tests on patients with peripheral vascular disease.

1003537411

CROSS REFERENCE SHEET

Name or Subject

Philip L. Woolf

Regarding

Research Grant #93

SEE

Richard L. Wechsler

1003537412

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

Application For Research Grant

#161
(Gr. #97 activated
7/1/55 and renewed
7/1/56).

Date: May 29, 1957

1. Name of Investigator: J. Edwin Wood
2. Title: Instructor in Medicine
Boston University School of Medicine
3. Institution & Address: Massachusetts Memorial Hospitals
Evans Memorial
65 East Newton Street
Boston 18, Massachusetts
4. Project or Subject: The acute effect of inhalation of tobacco smoke and of a cool environment upon reactive hyperemia blood flow of the calf segment of normal individuals and patients suffering peripheral vascular disease of this muscular segment.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

The purpose of this study would be to determine whether or not the depressant effect of tobacco smoking and of a cool environment upon reactive hyperemia of the skin (foot) also occurs in the limb segment. Of particular interest in this regard would be the patients who suffer intermittent claudication and who are relieved by sympathectomy. Since this depressant effect upon reactive hyperemia is apparently mediated via the sympathetic nervous system, the question arises as to whether or not the beneficial effects of sympathectomy upon intermittent claudication have to do with these stimuli interfering with removal of the products of metabolism.

The protocol of the experiment will be essentially the same as that used for the studies of foot circulation except that plethysmographic studies of the calf segment with arterial occlusion at the ankle will be used. The subject will be exposed to a room temperature of 83 degrees for a period of one hour or whatever period is required to achieve basal blood flows, following which a 15 minute control period of blood flows will be obtained. Arterial occlusion of 5 minutes duration followed by measurement of blood flows at short intervals will be carried out. The patient will then smoke two cigarettes at his own rate and the above experiment will be repeated during the course of his smoking the two cigarettes. Methods of measuring blood flow and analysis of results will be the same as that utilized in studies of limb segment reactive hyperemia described by Wood et al, Circulation Research 3: 581, 1955. In essence the method reveals the quantity of blood which passes through the limb in the immediate post-ischemia period.

1003537413

6. Budget Plan:
Salaries for technical
and secretarial assistance
\$2,500.

Salaries
Expendable Supplies
Permanent Equipment
Overhead (10.6%)
Other

\$2,500
300
300
300
\$3,100

Total

7. Anticipated Duration of Work: One year

8. Facilities and Staff Available: Facilities available remain the same as before; namely, constant temperature room with all the necessary plethysmographic and recording equipment. Project to be directed by Dr. J. Edwin Wood. Dr. Jay D. Coffman who has devoted a portion of his time to this project during the tenure of the present TIRC grant would continue as a part-time investigator in this program. Miss Barbara Sears who has participated as a technical assistant during the course of the first two TIRC grant years will continue in that capacity during the third year. Dr. Robert W. Wilkins, Director of the Cardiovascular Division, Evans Memorial, will be available at all times for consultation with regard to this project.

9. Additional Requirements: No additional requirements

10. Additional Information (Including relation of work to other projects and other sources of supply):

Results of previous studies carried out under the auspices of the TIRC have been published under the title "Comparative vasoconstrictor effects of inhaling tobacco smoke in warm and cool environments and before and after abstinence from tobacco," by Bohstein, J. W., Wood, J. E., and Wilkins, R. W., American Heart J. 53: 435, March 1957. Also, in abstract form, results of studies carried out during the present grant year are reported in the Proceedings of the American Society for Clinical Investigation under the title "Reduction of reactive hyperemia blood flow of the foot by tobacco smoking," by Wood, J. E., and Coffman, J. D.

1003537414

Signature /s./ J. EDWIN WOOD

Director of Project J. Edwin Wood

/s./ WALTER G. LAMB, M.D.

Business Officer of the Institution

Walter G. Lamb, M.D. Assistant
Administrator

History

Other
Overhead
Permanent Equipment
Expandable Supplies
Salaries

and Developmental Assistance
Bureau for Technical
Cooperation
Budget Plan

3. Anticipated Duration of Work:

8. Zeitliche Begrenzung

Facilities and Staff Available

The following facilities are available at the site:

- A large hall suitable for meetings and lectures.
- A kitchen and dining area.
- A bathroom.
- A storage room.

The staff consists of the following personnel:

- A Director.
- An Assistant Director.
- A Secretary.
- A Cook.
- A Cleaner.

The site is located in the center of the town and is easily accessible by public transport.

2. Additional Requirements:

Additional information (including relation of work to other projects and other sources of supply):

[illegible]

Director of Project

U.S. DEPT. OF JUSTICE

Business Officer of the Institution

1003537415

Committee:
Wilson
Cattell
Little

TOBACCO INDUSTRY RESEARCH COMMITTEE

#279

150 East Forty Second Street

New York 17, N.Y.

Application for Research Grant

Date: May 25, 1960

1. Name of Investigator: Summer Wood, Jr. with James L. Talbert and William F. Rienhoff, Jr.
2. Title: Assistant Professor of Pathology (S.W., Jr.), Resident and Fellow in Surgery (J.L.T.) and Associate Professor of Surgery (W.F.R., Jr.).
3. Institution & Address: Departments of Pathology and Surgery
Johns Hopkins University
Baltimore 5, Maryland
4. Project or Subject: Evaluation of etiologic factors, such as occupational hazards and habits, and pathologic peculiarities in the long-term survival of patients following resection of bronchogenic carcinoma.
5. Detailed Plan of Procedure: Since the initial pneumonectomy for bronchogenic carcinoma in 1933 at the Johns Hopkins Hospital (by Dr. W.F. Rienhoff, Jr.), more than 450 curative resections have been performed. From these cases there have emerged a significant group of 5-, 10-, and 20- or more year survivors, totaling at least 40 patients, that warrant careful analysis.

In the present investigation, a review of possible etiologic agents, such as occupational hazards and habits, as well as the pathologic details of this group in comparison with the short-term survivors is planned. The features to be individually analyzed include occupational hazards, habits, age, sex race, location, size, histologic type, lymph node metastasis, venous invasion and other pathologic findings such as in situ carcinoma (especially at the proximal bronchial margin), desmoplastic reaction within or about tumor, and adjacent inflammatory processes.

Representative pathologic material is available from virtually all of these resections performed at the Johns Hopkins Hospital. In addition to the clinical follow-ups and analyses of possible etiologic factors, extra sections will be prepared for the evaluation of microscopic features such as metastasis, histologic type and grade, and the degree and type of vascular invasion. The latter will be accomplished with the comparison of consecutive sections stained with Hematoxylin and Eosin and Verhoeff's technic for the demonstration of elastica within venous or arterial walls.

1003537416

TIRC
Appl. #279

6. Budget Plan:

Salaries	\$ 5100.00
Expendable Supplies	540.00
Permanent Equipment	2000.00
Overhead (15%)	1146.00
Other	-----
<hr/>	
Total	\$ 8786.00

7. Anticipated Duration of Work: 18-24 months

8. Facilities and Staff Available: Clinical records.
Tissue for histologic study.
Part-time secretary for follow-up.
Part-time secretary for indexing, coding, filing and analysis.
Part-time technicians for preparation of routine and special stains.
Adequate space.

9. Additional Requirements: None except permanent equipment and salaries for part-time secretarial and technical assistance. The salaries include \$1500 for part-time tissue technicians; and \$3600 for part-time secretaries for indexing, analysis, filing, follow-ups and coding. Permanent equipment required totals \$2000 for file, microtome, record copier and microscope.

10. Additional Information (Including relation of work to other projects and other sources of supply):

There are no other sources of financial support for this investigation. The significance and individuality of this project is provided by the availability of patients surgically "cured" of bronchogenic carcinoma of 5 to 28 years. The analysis of these cases may provide clues to the possible role and significance of etiologic factors, such as habits and occupational hazards, as well as the biologic behavior of pulmonary carcinoma, such as tumor type, grade, vascular (venous invasion) and metastasis. From preliminary data, it is apparent that certain cases of bronchogenic carcinoma are curable and that a small group may have useful survival periods of 10 years or more, although in some cases the malignant disease had not been completely eradicated. Because of the differing response of individuals to bronchogenic carcinoma, the realization that even lung cancer may behave as a chronic disease, and the rare possibility of spontaneous remission or regression with this form of malignancy, a critical evaluation of possible etiologic factors and the biologic behavior (especially vascular invasion, the major mechanism of peripheral metastasis) is proposed. At a future date, from the analysis of these and related data an experimental and clinical approach to the control of etiology and biologic behavior of lung cancer may emerge.

/s/ Summer Wood, Jr., Director of Project

/s/ Samuel P. Asper, Jr.
Business Officer of the Institution

1003537417

THE TOBACCO INSTITUTE, INC.

SUITE 1017, BARR BUILDING, 910-17TH STREET, N. W.

WASHINGTON 6, D. C.

EXECUTIVE LETTER

June 14, 1960

To: Members of The Tobacco Institute, Inc.
From: Edward F. Ragland
Subject: Massachusetts Labeling Bills

Late yesterday afternoon Mr. John Griffin of the J. P. Manning Company, Boston, Massachusetts, telephoned me to say that the two labeling bills which had been before the state legislature of Massachusetts have been killed for this session.

E.F.R.

1003537418

*current list
General files*

June 15, 1960

MEMORANDUM

TO: The Scientific Advisory Board

FROM: Robert C. Hockett

SUBJECT: Research Proposal (#279) from Sumner Wood, Jr.,
James L. Talbert and William F. Rienhoff, Jr.

This proposal was handed to the Associate Scientific Director during the course of the last meeting of the Scientific Advisory Board on May 26, 1960. It was not discussed at the meeting because copies were not ready for distribution.

The proposal has subsequently been studied by Dr. C. C. Little, E. B. Wilson and M. Cattell. These members of the Board consider the project so directly relevant and so timely that they have suggested that action be taken immediately by mail vote without waiting for the next meeting of the Board in late September.

Will you kindly drop me a line at your early convenience to register your opinion?

R.C.H.

Note: A minor modification is being suggested in line with general policy, to remove the item for Permanent Equipment from the total used as basis for the Overhead calculation. This will reduce Overhead to \$846.00 and the total to \$8,486.00. It will also be ascertained whether the budget proposed is expected to cover the entire 18-24 month period of study.

R.C.H.

RCH:mr
Encl.

1003537419

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street New York 17, New York

Application for Research Grant

#238

Date: May 21, 1959

1. Name of Investigator: John P. Wyatt, M.D.
2. Title: Professor of Pathology
3. Institution St. Louis University School of Medicine
& Address: 1402 S. Grand Boulevard, St. Louis 4, Missouri
4. Project or Subject: An Investigation into the Nature of the Pigmentary Lesion in Centrilobular Emphysema.
5. Detailed Plan of Procedure:

Utilizing the whole lung paper section technic¹ stereoscopic and histologic methods, a current morphologic analysis has revealed a distinctive anatomic form of emphysema, characterized by its early lesions developing in the central lobular portion of Miller's secondary lobule.² This distinctive form of emphysema is readily separated from the traditional obstructive vesicular emphysema with the lesion being at the respiratory bronchiole of orders 1 and 2.^{3,4}

From our analysis to date, an outstanding structural change, associated with this respiratory bronchiolar lesion in centrilobular emphysema has been remarkable accumulation of black pigment. Although this black pigment is probably of exogenic derivation, studies of a definitive nature, concerning the origin, chemical and physical makeup, and the possible morphogenetic role of this pigment in centrilobular emphysema have not been undertaken previously.

It is our proposal, with micro-thin whole lung preparations and stereoscopy as aids in the identification and orientation of the respiratory bronchioles, to investigate more closely with precise micro dissection the physical and chemical nature of this "pigmentary" lesion by micro-incineration, volatility, fluorescence, and chromatographic studies.

As a complementary procedure to this physical and chemical analysis of the altered respiratory bronchioles, it is proposed with serial histologic sections to investigate in human tissues the existence and histologic makeup of the "sump pumps"⁵ which apparently have their localization, anatomic and functional, at the 1st and 2nd respiratory bronchioles, the anatomic site of centrilobular emphysema. With this approach further insight into the neglected problem of the lung toilet and dust disposal may be gained.

6. Budget Plan:

Salaries	5,400.00
Expendable Supplies	920.00
Permanent Equipment	370.00
Overhead (15%)	1,003.00
Total	\$7,693.00 p.a.

1003537420

7. Anticipated Duration of Work: 2 years

8. Facilities and Staff Available:

Complete Pathologic Laboratory, including: Macro-section microtome (Wentworth type); Histochemical Laboratory, Fluorescent microscopy, Chemical Laboratory for Mineralogical Study; Chromotographic and Electrophoretic Apparatus.

Full-time Ph.D. Biochemist in Department of Pathology, as consultant to this project.

9. Additional Requirements:

Contemplate 2 additional technicians, one in morphology, one in biochemistry.

10. Additional Information:

Functional and Pathological Correlatives of Emphysema presently being supported by U.S.P.H.S. Grant. This grant H-3535 is primarily concerned with quantitating emphysema and adaptive changes as observed at postmortem with clinical aspects of the disease.

Signature John P. Wyatt, M.D.
Director of Project

James W. Colbert Jr., M.D.
Business Officer of Institution

References

1. Gough, J. The Use of Thin Sections of Entire Organs in Morbid Anatomical Studies. J. Roy. Micro. Soc., 69:231-235, 1949.
2. The Pathology of the Pneumoconiosis in Welsh Coal Miners. Proc. Int. Cong. in Ind. Med. Ed. 9. 661-667, 1948.
3. Wyatt, J. P. Macrosection and Injection Technics in Emphysema. 1st Internat. Conference at Aspen, Colorado, June 12-14, 1958. Published in July Issue of American Review of "Tuberculosis and Pulmonary Disease," July 1, 1959.
4. Centrilobular Emphysema. American Association of Pathologists and Bacteriologists, April 23, 1958, Boston, Mass.
5. Macklin, Charles. Pulmonary Sumps, Dust Accumulations, Alveolar Fluid and Lymph Vessels, Acta Anatomica, 23:1-33, 1955.

1003537421

STANDARD B & P "NOTAR"

STANDARD B & P

1003537422

X YZ

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

Application For Research Grant

121

Date: December 23, 1955

1. Name of Investigator: Oleg Yadoff

2. Title: President and Scientific Director

3. Institution: Foundation for Bio-Physico Research
& Address: 215 Coleridge Avenue, Palo Alto, California

(This is a non-profit corporation of the State of New York, incorporated Nov. 10, 1953)

4. Project or Subject: Experimental research on the effect of tobacco, in particular of nicotine, on tumor cells and cancer formation in small tropical Guppies, an aquarium-raised marine animal especially selected for this purpose.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed): The above-described study is of particular interest because of the fact that Guppies in the normal state are particularly susceptible to breast tumors, the incidence of this affliction amongst them being in the neighborhood of 95%. This ailment may therefore be said to constitute a factor helping to determine their life-span, cutting off their lives at the peak. Two to three weeks before finally succumbing to it they are still capable of reproduction. One might say that because of it Guppies die in the flower of adulthood without ever growing old. The creation of a biological environment provided with certain elements ordinarily lacking in the Guppies' natural environment is the first step in reducing the incidence of the tumors. The second is a rationalized diet, compact of those elements most favorable to growth and continuing health. Said diet includes vitamins, minerals, metals. Metals such as iron, copper, magnesium, greatly affect growth and mutations in Guppies. Their controlled introduction into the Guppies' rationalized diet makes it possible to produce marked mutations, assuredly due to the change of the hormones and as a function of the latter to the change of the physiological state of the fish. The increased growth of tails and fins shows the effect of regeneration by differentiation. Which gives some idea of the exceptional usefulness of Guppies in studying the effect of tobacco, and in particular of nicotine.

The Plan of Procedure involves the following: To secure suitable aquarium tanks, capacity being one gallon of water per pair of fish, wherein the proper biological environment (determined in previous studies) would be maintained throughout. Experimental fish would be all of the same age, the youngest, for example, being all 3 months old, the next youngest 6 months, the next 1 year, the next 1½ years, etc. Captured smoke and fumes from cigarettes and cigars would be infused into the water in the tanks in regulated doses.

1003537423

No. 5 (continued)

It is known that smoke is not disintegrated by water but is conducted by it. However, the nicotine in the smoke will be strained out and will remain in the water, polluting it more and more, in proportion to the smoke doses administered. The fish would be exposed to the water thus polluted for varying controlled periods - one hour, two hours, three hours, etc. ...one day, two days, three days, etc. ...one week, two weeks, etc., samples all along being subjected to chemical and biological tests and analyses and microscopic inspection.

We have immediately available three to four thousand of these Guppies ready for such experiments. Each fish would be examined anatomically and chemically. Thus, the main part of our research would be on the possibility of a connection between nicotine and cancer.

Supplementary studies could be run wherein fish would be subjected simultaneously to the effects of nicotine and antibiotic foods, such as Aminopterin and Actinomycin, which have already been used successfully.

This project would require my own constant presence in the laboratory as well as that of an assistant physicist, part-time, and assistant biologist, part-time, and for the preparation of reports, possible articles and general administration work of an editor-secretary, also part-time. The said project would require one year.

1003537424

6. Budget Plan:

Salaries	\$ 6,000
Expendable Supplies	2,800
Permanent Equipment	1,300
Overhead	1,000
Other	1,000
Total	\$12,100

7. Anticipated Duration of Work:

One year

8. Facilities and Staff Available:

One laboratory comprising two rooms, one 30' x 14', the other 18' x 14', plus auxiliary rooms, all equipped with needed lighting, water supply, heating, tank capacity, smoke injectors and pumps, chemical and biological materials required, and 3 to 4 thousand selected Guppies.

9. Additional Requirements:

To have built two tanks, one of 150 gallons, the other of 60 gallons; installation of electrical apparatus for pretreatment of water; biological microscope; surgical instruments; air-compressor (5 atmospheres); various feeds.

10. Additional Information (Including relation of work to other projects and other sources of supply):

Experimental studies on antibiotic methods, with use of Aminopterin and Actinomycin, are being conducted with great success under the direction of Dr. Selman A. Waksman at the New Jersey Agriculture Experimental Station and at the Microbiology Laboratory of Rutgers University. The undersigned believes that an exchange of views with the aforementioned would be most useful.

Signature s/ Oleg Yadoff
Director of Project

s/ Oleg Yadoff, President
Business Officer of the Institution

1003537425

TOBACCO INDUSTRY RESEARCH COMMITTEE

Committee:
S.A.B.

150 East Forty Second Street

#257

New York 17, N.Y.

Application For Research Grant

Date: October 8, 1959

1. Name of Investigator: George S. Zuccala B.S. Sc.D. F.A.C.M.T.
2. Title: Chief Serologist and medical technologist.
3. Institution & Address: National Research Foundation, Inc. & Zuccala Research Lab., Inc.
179 Allyn St., Hartford, Conn; Jackson 2-4895 - Adams 3-0508
4. Project or Subject: The effect of smoking on the human body sera, and vital organs traced by means of blood test, urinalysis and saliva.

To note if cigarette tars is cancer motivating agent or the bacteria toxicity who might be the cause of such claim made by other scientists who stated that cigarette tar is cancer motivating agent.

If tar is cancer motivating agent, a substances are made to overcome such reaction, as matter of fact even cancer prevantative agent can be made by means of an antigen.

5. Detailed Plan of Procedure: Take one thousand people from different parts of United States who are in the habit of constant smoking. This blood and urine specimens are collected through doctor's office, like is done in collecting specimens for diagnostic test.

Take one thousand people of age and sex who do very little smoking, but note the place of working, to note if cigarette smoke or gas fumes may cause a certain "Lytic" reaction in the bloodstream.

Take one thousand people who live in city and one thousand in farms and open air.

Then make correlation and calculations if this blood, urine and saliva tests if necessary if "Lytic" are caused by smoke, if so what counter-reactor can we find?

Since the blood is the only liquid that travels throughout the entire human body, any physical or chemical changes caused by smoke or food or bacteria, virus etc., must be noticed in the human body sera. Very little is known about serology and the effect of smoke and foods.

This program is a history-making program which may reveal many things, and tobacco can be used as beneficial to a certain health condition, as well to release headaches.

This is a very unusual program, never attempted before, because we have learned very little about natural antibodies and natural complement and the effects and reaction of body sera.

1003537426

6. Budget Plan:

Depends on the number of positive
"Lytic" reaction are found on
people heavy smoking.

Salaries	\$ 16,840.
Expendable Supplies	2,000.
Permanent Equipment	1,500.
Overhead	2,400.
Other (traveling and mail)	2,000.
	<hr/>
	\$24,740.

7. Anticipated Duration of Work:

About one year

8. Facilities and Staff Available:

We have staff and facilities and cooperation from the medical profession
as licensed clinical diagnostic laboratory.

9. Additional Requirements:

None, unless the Tobacco Industry Research Committee wish us to go further,
after we make our reports of our findings, in connection of smoking and blood
serological changes.

10. Additional Information (Including relation of work to other projects and
sources of supply):

We might seek the cooperation of every State Dept. of Health in United States to
supply us with human blood sera, after they are finished with their daily routine,
by obtaining such specimen, the work will be done in very large-scale and better
information will be obtained of the number of positive "Lytic" reaction are reported,
and then survey if these positive cases are heavy smokers, or are connected with
industries where gas fumes are responsible for cancer of the lungs or the cigarette
tar, as Sloan-Kettering Institute claims. There is a way to neutralize the cigarette
tars in order not to cause cancer or other ill effects. One poison can destroy the
other, the same we can change acid into alkaly. This work is done by qualified
serologist only, and trained technicians in such field.

/s/ George S. Zuccala, Sc.D.
Director of Project

/s/ Joseph M. Rossi, B.A.
National Research Foundation, Inc.
and Zuccala Research Lab., Inc.

1003537427

Applicants for

Research Grants

1003537428

NAME OF INDIVIDUAL INVESTIGATOR AND INSTITUTION

APPLYING FOR GRANTS FROM TIRC

1003537429

<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
1	WILEY, Richard H., Prof. & Chairman, Chemistry Dept.	University of Louisville, Belknap Campus	Reformation of the nicotine molecule.	60,320	6/14	
2	SEGAL, Maurice S. M.D., Dir. Dept. of Inhalational Therapy, Boston City Hospital	Tufts College Medi- cal School - Boston City Hospital	Effects of cigarette smoking on normal subjects.	22,000	6/19	
3	WASE, A. W., Ph.D., Asst. Prof. of Bio- logical Chemistry	Hahnemann Medical College & Hospital of Philadelphia	Biochemistry of Pulmonary tissue as influenced by tobacco smoke.	7,100	6/16	
4	WENDER, Simon H., Res. Prof. of Chem- istry	University of Okla- homa Research In- stitute	A qualitative & quantitative study of the individual polyphenol content of cigarette tobacco & of the cigarette smoke, & also to study the fate of these compounds in the animal respiratory system.	12,400	6/14	
5	COBE, Dr. Herbert M., Prof. Microbiology & Bacteriology	Temple University	The effect of tobacco & other con- stituents (chemical compounds) on the bacterial flora of the oral cavity & the respiratory passages.	12,200	6/16	
6	SALTMAN, Paul D., Ph.D., Assistant Professor	University of Southern Califor- nia	The enzymatic mechanism for the dark fixation of CO ₂ by tobacco	7,776	6/26	
7	GRIFFIN, Dr. A. Clark, Associate Professor of Biochemistry	Stanford University Chemistry Dept.	The effect of exposure to cigarette smoke on the induction of cancer by chemical compounds.	5,960	6/21	
8	MANN, Dr. David E., Associate Prof. of Pharmacology	Temple University School of Pharmacy	Effect of tobacco smoke and tobacco residues on methylcholanthrene-induced skin carcinogenesis in mice.	5,500	6/24	
9	GOODSON, Louis H., Ph.D., Sr. Research Chemist	Midwest Research Institute	Study of lung tissue changes produced by air pollutants including tobacco smoke.	47,000	7/2	

1003537430

<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
10	AYRE, J. Ernest, M.D., Director	The Cancer Institute at Miami	The systematic study of possible carcinogens in cigarette paper tar.	21,000	7/2	
11	FITZGERALD, Dr. P. J., Prof. & Executive Head	State University of N. Y. College of Medicine	A study of the incidence of carcinoma <u>In Situ</u> of the lung in autopsies of males over 30 years of age.	7,625	7/7	
12	FREEDLANDER, B. L., M.D., Director of Cancer Research	Mt. Zion Hospital	The proposed research on experimental mouse cancer may be divided into the following three projects: (are divided on application blank)	8,900	7/7 10/9 (amended app.)	
13	HOLDEN, Dr. Frances R., Senior Physical Chemist	Stanford Research Institute	The physico-physiological properties of tobacco smoke.	78,600	7/21	
14	MOTLEY, Hurley Lee, M.D., Prof. of Medicine	University of Southern California School of Medicine	A study of the effects of smoking on pulmonary function.	31,000	8/4	
15	WOERNER, Charles Arthur, Ph.D., M.D., Asso. Prof. of Anatomy	University of Louisville, School of Medicine	A study of the effect of Tobacco tars and extracts on the arteries of experimental animals.	9,900	8/2	
16	WELLER, Russell W., M.D., Associate Prof. of Pathology	Hahnemann Medical College & Hospital of Phila.	A postmortem study of the bronchi, lungs and heart correlated with inhaled substances related to occupation, residence and smoking.	8,181	8/2	
17	BAILEY, Paul C., Professor of Biology	Alabama College	A study of the effects of tobacco smoke upon growth and cell division in: a. root tips of <u>Trillium sessile</u> L. and b. the chick embryo	1,600	8/4	
18	LOBSTEIN, Otto E., Director of Research	Chem-Tech Laboratories	The effect of enzymes on the growth of lymphosarcoma. 1. The role of lysozyme 11. The role of other enzymes, proteolytic, mucolytic, and others.	7,500	8/6	

1003537431

<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
19	MONTGOMERY, Philip O'Bryan, Asso. Prof., Pathology	The University of Texas, South- western Medical School	The investigation of the possible role of chronic inflammation in chemical carcinogenesis.	5,400	8/10	
20	STARE, Fredrick J., Ph.D., Prof. of Nutrition	Harvard School of Public Health, Dept. of Nutrition	Experimental studies on cancer util- izing a new technique to see if vari- ous tars extracted from tobacco may incite the formation of lung tumors.	13,613	8/12	
21	JACOBS, William Lee	Independent Investigator	(briefly) To show the relationship of lung irritation and cancer to the use of lighting agents.	5,700	8/11	
22	LIKES, Dr. Carl J., Project Supervisor	Virginia Institute for Scientific Research	Metabolism and Catabolism of leaf proteins in tobacco (<u>Nicotiana</u> <u>tabacum</u> .)	15,500	8/18	
23	GROSSE, Dr. A. V., Dir. of Project	Research Institute of Temple Univer- sity	Research on the chemistry of cigarette smoking which should provide new infor- mation regarding the effect on health of cigarette smoking and possible improve- ments in the composition of cigarettes if, shown to be necessary.	82,250 (2 yrs.)	8/22	
24	HAAG, H. B., M.D., Prof. of Research Pharmacology	Medical College of Virginia	Preparation for publication of a book on the biologic aspects of tobacco and its smoke.	33,990	8/25	
25	HAWTHORNE, Herbert. R., M.D., Chairman, Prof. of Surgery	University of Pennsylvania, Dept. of Surgery, Grad. School of Medicine	The production of induced pulmonary neoplasms in experimental animals by exposure of the Tracheo-bronchial system to tobacco smoke.	36,300	8/27	
26	SHULMAN, Maurice H., Principal Investigator	Boston University, Dept. of Biology, Graduate School	Direct observations on blood vessels during exposure to the constituents of cigarette and pipe smoke.	45,028	9/9	

1003537432

<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
27	MC KEE, Kelly, T., M.D., Associate Prof. of Medicine	Medical College of South Carolina	Study of lung function in smokers and non-smokers.	7,900	9/12	
28	MOORE, George, E., M.D., Ph.D.	Roswell Park Mem- orial Institute	An investigation of the physiological effects of direct inhalation of tobacco smoke by laboratory animals and the study of the biological response of laboratory animals to continuous ingestion of diet- tobacco product mixtures.	30,542.40	10/1	
29	HOMBURGER, F. M.D., Director	Tufts College Medical School, Dept. of Surgery	Effects of various components of tobacco and cigarette paper upon the behavior of transplantable tumors in various species including the behavior of human tumors transplanted into animals.	126,630	8/23	
30	SCHEPERS, G. W. H., M.D., D.Sc., Director	The Saranac Laboratory	Environmental Pulmonary Carcinogenesis. The co-carcinogenic potentialities of inhaled tobacco smoke in relation to beryllium-provoked lung cancer of the rat.	49,356	10/4	
31	CLARKE, Hans T., Professor of Bio- chemistry	Columbia Univer- sity, College of Physicians and Surgeons	Biochemistry of White Blood Cells. 1. Proteolytic activities of the white blood cells of man and the effect on white blood cell activities of carcino- gens, nutrition, and other influences.	19,958	10/8	
32	CERECEDO, Leopold R., Professor of Biochemistry	Fordham Univer- sity	A study of early chemical changes in the lungs of tumor-bearing rats and mice.	8,360	10/7	
33	SULZBERGER, Marion B. Prof. & Chairman, Dept. of Dermatology & Syphilology, N.Y.U. Post-Graduate Med. School & Dir., N. Y. Skin Cancer Unit	New York Univer- sity, Bellevue Medical Center	Investigation of the effects of tobacco on the human vascular system in living volunteers; and in particular of the possibility that certain tobacco effects are based on peculiar allergic suscepti- bility of specific individuals rather than upon obligatorily toxic products in tobacco smoke.	15,000 (per annum)	11/8	

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<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
34	McLAUGHLIN, John T., M.D., Dir. of Bio- physics Research	Institute of Nuclear and Atom- ic Sciences	Analysis of tobacco for radioactiv- ity...a qualitative determination by means of mass spectroscopy. A preliminary survey to determine the feasibility, mechanics and cost of such analyses.	2,139.50	10/12	
35	IUISADA, Aldo A., M.D., Director	The Chicago Medi- cal School, Div. of Cardiology	Action of products of combustion of tobacco leaves on the circulation.	10,340	10/13	
36	WOLFF, William A., A.M., Ph.D., Associate Prof. of Clinical Chem- istry & Toxicology	Bowman Gray School of Medicine	<u>Project A:</u> The Fate of Tars from Cigarette Smoke Deposited in the Dog Lung - <u>Project B:</u> Cigarette Smoke in the Human Lung, A Radioisotope Study	25,000	10/11	
37	REVICI, Emanuel, M.D., Scientific Director	Institute of Ap- plied Biology	To determine whether tobacco smoke produces the nonspecific, abnormal metabolic pattern found by us in sus- ceptible animals and humans, which may influence the evolution of pre-cancer- ous or non-invasive cancer cells or other abnormal tissues.	12,000	10/22	
38	GOLDSTEIN, Dr. Jacob, Associate, Workshop in Sociological Res. Tech- niques, Grad. Faculty	New School for Social Research	An exploratory study of personality correlates of cigarette smoking among males in the 40-and-above age group.	23,500	10/29	
39	VOUGHT, Robert L., M.D., Associate Prof. of Epidemiology	Columbia Univer- sity, School of Public Health	The Design for a Long Term Study of Hypertension.	25,322	11/1	
40	BENHAM, G. H., Super- visor, Biochemistry Section	Armour Research Foundation	Does tobacco smoke elicit a stress reaction?	20,000	11/2	

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<u>Nó.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
41	<u>BORDENCA</u> , Dr. Carl, Asst. Head, Organic Division	Southern Research Institute	The study of preferential combustion or oxidation of cigarette components during smoking.	36,000	11/8	
42	<u>HEATH</u> , Clark W., M. D.	Harvard University, Department of Hygiene	Personality and smoking in college graduates: a fifteen-year follow-up study.	15,880	12/10	
43	<u>WILSON</u> , Robert C., Associate Professor & <u>SQUIER</u> , Leslie H., Instructor	Reed College, Psychology Department	A study of the relationships between personality patterns, smoking behaviors and lung cancer, cancer, and heart diseases.	41,712	11/15	
44	<u>KOTIN</u> , Paul, M. D., Assistant Professor of Pathology	University of Southern California Medical School	The experimental production of carcinogenic hydrocarbons in simulated cigarette smoking.	7,560	11/12	
45	<u>ABRAMS</u> , Arnold, Ph.D., Research Scientist (Psychology)	Syracuse University Research Institute, Psychology Department	An epidemiological study of lung cancer and its relationship to certain sociopsychological factors.	96,500	11/19	
46	<u>WAGNER</u> , Bernard M., M. D.	Hahnemann Medical College & Hospital	Relationship of tobacco products to vascular disease.	12,420	11/25	
47	<u>PICKEL</u> , Frank D., Ph.D.	Evans Research and Development Corp.	Chemical studies of tobacco, tobacco additives and cigarette smoke.	30,125	11/26	
48	<u>SIEGEL</u> , Arthur I., Dr., Director	Applied Psychological Services	The need for the proposed series of investigations into the effects of tobacco on various sensory & motor processes is partially summarized in the recent <u>Tufts College Handbook of Human Engineering Data</u> which states, "although tobacco is frequently cited as a possible contributory factor in numerous medical disorders of the sensory processes, <u>the literature is practically devoid of objective studies on the influence of tobacco alone on the sensory mechanisms of normal individuals. Research in this area is greatly needed.</u> " (emphasis ours)	12,180	11/30	

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<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
49	KNUDTSON, Kenneth P., Clinical Assistant Professor of Pathology	University of Washington, Medical School	A pathologic and topographic study of bronchial mucosa with special refer- ence to the relationship of squamous metaplasia, atypical epithelial pro- liferation and bronchogenic carcinoma in smokers and non-smokers.	5,400	12/5	
50	<u>PATHOLOGIC-ANATOMIC SURVEY</u>	A number of institutions	Pathologic-Anatomic study of cellular changes in human bronchi.	55,000		
51	PRATT-THOMAS, H. R., M.D., Professor of Pathology	Medical College of South Carolina	Biological assay of cancer producing factors in cigarette smoke tars.	8,134.50	12/3	
52	MURRAY, William S., Sc.D., Research Asso- ciate & Administration Director	Roscoe B. Jack- son Memorial Laboratory	The production of genetically controlled animals and tumors for use in experimental research on tobacco in relation of health by (a) the expansion of known inbred stocks and sources of tumor supply; (b) the pro- duction of such hybrids or heterozygous types as become necessary.	47,318	12/20	
53	MONTGOMERY, Hugh, M.D., Associate Professor of Medicine	University of Pennsylvania Medical School	Influence of tobacco smoking on the blood flow of skin and of muscles of extremities in sympathectomized and un- sympathectomized subjects.	10,667.50	12/22	
54	BARNES, Frederick W., Jr., M.D., Ph.D.	The Johns Hopkins University School of Medicine	The role of hyperplasia in tissue re- sponse to chronic damage.	11,000 (per yr. for 3 yrs.)	1/4/55	
55	BARACH, Alvan L., M.D. Clinical Professor of Medicine	Columbia Univer- sity, College of Physicians & Surgeons	Effect of hypoxia on tumor growth in animals protected by induced hypothy- roidism.	17,560	1/7	
56	VOLKER, Joseph F., D.D.S., Ph.D., Dean	University of Alabama, School of Dentistry	The effects of tobacco on selected oral structures.	31,000	1/7	

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<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
57	STALLWORTH, J. Manly, M.D.	Medical College of South Carolina	The effects of cigarette smoke on the peripheral vascular system.	4,104	1/13	
58	HARKAVY, Joseph, M.D.	The Mount Sinai Hospital	Role of tobacco in cardio-vascular disease.	11,625	1/19	
59	COOPER, Philip, M.D., Chief, Surgical Service Dir., Surgical Research Laboratory	Veterans Admin- istration Hos- pital	A study of the effects of cigarette smoking on levels of gastric acid and pepsin. Effect of smoking on levels of uropepsin will also be investigated.	15,000	1/25	
60	CLINE, Joseph K., Ph.D. Dir., Cancer Dept., Prof. of Experimental Chemistry	Medical College of Alabama	Quantitative study of the composition of tars produced from smoke of tobacco, cigarettes, cigarette paper with and with- out additives with special reference to carcinogenic hydrocarbons. Carcinogenic and co-carcinogenic effects in mice.	32,940	1/26	
61	MEISELAS, Leonard E. M.D., Clinical In- structor in Medicine	State University of N. Y., College of Medicine at New York City	1. To determine the metabolic pathways of compound E and compound F in the cancer patient, in the patient with heart disease and in the normal. 2. To determine whether Aldosterone is a normal or abnormal metabolic product of E and F. 3. To determine whether the presence of an abnormal function- ing liver is related to the produc- tion of Aldosterone. 4. To determine whether the presence of an abnormal functioning liver is related to the production of Aldosterone.	12,071	2/14 (revised)	
62	HAFKENSCHIEL, Joseph H., M.D., Director of Cardiopulmonary Unit	Lankenau Hospital	Measurement of coronary blood flow, cardiac work and cardiac oxygen and carbohydrate metabolism in normoten- sive subjects before and after intra- venous nicotine and after smoking standard cigarettes.	21,541 (two years)	1/28	
63	SIMON, David L., In- structor in Medicine	University of Cincinnati	The effects of chewing tobacco on the cardiovascular system of man.	2,800	2/7	

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No.	Investigator	Institution	Subject	Amount	Date	Disposition
1	WILEY, Richard H., Prof. & Chairman, Chemistry Dept.	University of Louisville, Belknap Campus	Reformation of the nicotine molecule.	60,320	6/14	
2	SEGAL, Maurice S. M.D., Dir. Dept. of Inhalational Therapy, Boston City Hospital	Tufts College Medi- cal School - Boston City Hospital	Effects of cigarette smoking on normal subjects.	22,000	6/19	
3	WASE, A. W., Ph.D., Asst. Prof. of Bio- logical Chemistry	Hahnemann Medical College & Hospital of Philadelphia	Biochemistry of Pulmonary tissue as influenced by tobacco smoke.	7,100	6/16	
4	WENDER, Simon H., Res. Prof. of Chem- istry.	University of Okla- homa Research In- stitute	A qualitative & quantitative study of the individual polyphenol content of cigarette tobacco & of the cigarette smoke, & also to study the fate of these compounds in the animal respiratory system.	12,400	6/14	
5	COBE, Dr. Herbert M., Prof. Microbiology & Bacteriology	Temple University	The effect of tobacco & other con- stituents (chemical compounds) on the bacterial flora of the oral cavity & the respiratory passages.	12,200	6/16	
6	SALTMAN, Paul D., Ph.D., Assistant Professor	University of Southern Califor- nia	The enzymatic mechanism for the dark fixation of CO ₂ by tobacco	7,776	6/26	Approved
7	GRIFFIN, Dr. A. Clark, Associate Professor of Biochemistry	Stanford University Chemistry Dept.	The effect of exposure to cigarette smoke on the induction of cancer by chemical compounds.	5,960	6/21	Approved
8	MANN, Dr. David E., Associate Prof. of Pharmacology	Temple University School of Pharmacy	Effect of tobacco smoke and tobacco residues on methylcholanthrene-induced skin carcinogenesis in mice.	5,500	6/24	Approved
9	GOODSON, Louis H., Ph.D., Sr. Research Chemist	Midwest Research Institute	Study of lung tissue changes produced by air pollutants including tobacco smoke.	47,000	7/2	

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<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
10	AYRE, J. Ernest, M.D., Director	The Cancer Institute at Miami	The systematic study of possible carcinogens in cigarette paper tar.	21,000	7/2	
11	FITZGERALD, Dr. P. J., Prof. & Executive Head	State University of N. Y. College of Medicine	A study of the incidence of carcinoma <u>In Situ</u> of the lung in autopsies of males over 30 years of age.	7,625	7/7	
12	FREEDLANDER, B. L., M.D., Director of Cancer Research	Mt. Zion Hospital	The proposed research on experimental mouse cancer may be divided into the following three projects: (are divided on application blank)	8,900	7/7 10/9 (amended app.)	<i>Approved</i>
13	HOLDEN, Dr. Frances R., Senior Physical Chemist	Stanford Research Institute	The physico-physiological properties of tobacco smoke.	78,600	7/21	
14	MOTLEY, Hurley Lee, M.D., Prof. of Medi- cine	University of Southern Califor- nia School of Medicine	A study of the effects of smoking on pulmonary function.	31,000	8/4	
15	WOERNER, Charles Arthur, Ph.D., M.D., Asso. Prof. of Anatomy	University of Louisville, School of Medicine	A study of the effect of Tobacco tars and extracts on the arteries of ex- perimental animals.	9,900	8/2	
16	WELLER, Russell W., M.D., Associate Prof. of Pathology	Hahnemann Medical College & Hospi- tal of Phila.	A postmortim study of the bronchi, lungs and heart correlated with in- haled substances related to occu- pation, residence and smoking.	8,181	8/2	
17	BAILEY, Paul C., Professor of Biology	Alabama College	A study of the effects of tobacco smoke upon growth and cell division in: a. root tips of <u>Trillium sessile</u> L. and b. the chick embryo	1,600	8/4	
18	LOBSTEIN, Otto E., Director of Research	Chem-Tech Laboratories	The effect of enzymes on the growth of lymphosarcoma. 1. The role of lysozyme 11. The role of other enzymes, proteo- lytic, mucolytic, and others.	7,500	8/6	

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<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
19	MONTGOMERY, Philip O'Bryan, Asso. Prof., Pathology	The University of Texas, South- western Medical School	The investigation of the possible role of chronic inflammation in chemical carcinogenesis.	5,400	8/10	
20	STARE, Fredrick J., Ph.D., Prof. of Nutrition	Harvard School of Public Health, Dept. of Nutrition	Experimental studies on cancer util- izing a new technique to see if vari- ous tars extracted from tobacco may incite the formation of lung tumors.	13,613	8/12	
21	JACOBS, William Lee	Independent Investigator	(briefly) To show the relationship of lung irritation and cancer to the use of lighting agents.	5,700	8/11	
22	LIKES, Dr. Carl J., Project Supervisor	Virginia Institute for Scientific Research	Metabolism and Catabolism of leaf proteins in tobacco (<u>Nicotiana</u> <u>tabacum.</u>)	15,500	8/18	
23	GROSSE, Dr. A. V., Dir. of Project	Research Institute of Temple Univer- sity	Research on the chemistry of cigarette smoking which should provide new infor- mation regarding the effect on health of cigarette smoking and possible improve- ments in the composition of cigarettes if shown to be necessary.	82,250 (2 yrs.)	8/22	
24	HAAG, H. B., M.D., Prof. of Research Pharmacology	Medical College of Virginia	Preparation for publication of a book on the biologic aspects of tobacco and its smoke.	33,990	8/25	Approved
25	HAWTHORNE, Herbert. R., M.D., Chairman, Prof. of Surgery	University of Pennsylvania, Dept. of Surgery, Grad. School of Medicine	The production of induced pulmonary neoplasms in experimental animals by exposure of the Tracheo-bronchial system to tobacco smoke.	36,300	8/27	
26	SHULMAN, Maurice H., Principal Investigator	Boston University, Dept. of Biology, Graduate School	Direct observations on blood vessels during exposure to the constituents of cigarette and pipe smoke.	45,028	9/9	

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<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
27	MC KEE, Kelly, T., M.D., Associate Prof. of Medicine	Medical College of South Carolina	Study of lung function in smokers and non-smokers.	7,900	9/12	
28	MOORE, George, E., M.D., Ph.D.	Roswell Park Mem- orial Institute	An investigation of the physiological effects of direct inhalation of tobacco smoke by laboratory animals and the study of the biological response of laboratory animals to continuous ingestion of diet- tobacco product mixtures.	30,542.40	10/1	
29	HOMBURGER, F. M.D., Director	Tufts College Medical School, Dept. of Surgery	Effects of various components of tobacco and cigarette paper upon the behavior of transplantable tumors in various species including the behavior of human tumors transplanted into animals.	126,630	8/23	
30	SCHEPERS, G. W. H., M.D., D.Sc., Director	The Saranac Laboratory	Environmental Pulmonary Carcinogenesis. The co-carcinogenic potentialities of inhaled tobacco smoke in relation to beryllium-provoked lung cancer of the rat.	49,356	10/4	
31	CLARKE, Hans T., Professor of Bio- chemistry	Columbia Univer- sity, College of Physicians and Surgeons	Biochemistry of White Blood Cells. 1. Proteolytic activities of the white blood cells of man and the effect on white blood cell activities of carcino- gens, nutrition, and other influences.	19,958	10/8	
32	CERECEDO, Leopold R., Professor of Biochemistry	Fordham Univer- sity	A study of early chemical changes in the lungs of tumor-bearing rats and mice.	8,360	10/7	
33	SULZBERGER, Marion B. Prof. & Chairman, Dept. of Dermatology & Syphilology, N.Y.U. Post-Graduate Med. School & Dir., N. Y. Skin Cancer Unit	New York Univer- sity, Bellevue Medical Center	Investigation of the effects of tobacco on the human vascular system in living volunteers; and in particular of the possibility that certain tobacco effects are based on peculiar allergic suscepti- bility of specific individuals rather than upon obligatorily toxic products in tobacco smoke.	15,000 (per annum)	11/8	

Approved

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TOBACCO INDUSTRY RESEARCH COMMITTEE

April 20, 1955

ACTIVE GRANTS

	<u>Total Value</u>	<u>Date Activated</u>
# 6 - Saltman, Paul D., University of Southern California	\$ 7,776.00	10/1/54
# 8 - Mann, David, Temple University, School of Pharmacy	5,500.00	10/1/54
#24 - Haag, H. B., Medical College of Virginia	33,990.00	11/1/54
#31 - Clarke, Hans T., Columbia University	19,958.00	11/1/54
# 7 - Griffin, A. Clark, M.D. Anderson Hospital, U. of Texas	5,960.00	12/1/54
#28 - Moore, George E., Roswell Park Memorial Institute	30,542.40	12/1/54
#12 - Freedlander, B. L., Mt. Zion Hospital	8,900.00	1/1/55
#20 - Stare, Fredrick J., Harvard School of Public Health	13,613.00	1/1/55
#32 - Cerecedo, Leopold, Fordham University	8,360.00	1/1/55
#42 - Heath, Clark W., Harvard University	15,880.00	1/1/55
# 2 - Segal, Maurice S., Tufts College Medical School	22,000.00	1/1/55
#25 - Hawthorne, Herbert R., University of Pennsylvania, Graduate School of Medicine	30,000.00	2/1/55
#27 - McKee, Kelly T., Medical College of South Carolina	7,900.00	2/1/55
#33 - Sulzberger, Marion B., NYU, Bellevue Medical Center	15,000.00	2/1/55
#50 - Pathologic-Anatomic Project (Cooperative)	81,000.00	2/1/55
#54 - Barnes, Frederick W., Jr., Johns Hopkins University School of Medicine	11,000.00	2/1/55
#44 - Falk, Hans L., University of Southern California School of Medicine	7,560.00	3/1/55
#52 - Murray, William S., Roscoe B. Jackson Memorial Lab.	43,112.00	3/1/55

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Active Grants

- 2 -

April 20, 1955

	<u>Total Value</u>	<u>Date Activated</u>
#53 - Montgomery, Hugh, University of Pennsylvania, Medical School	10,667.50	3/1/55
#62 - Hafkenschiel, Joseph H., The Lankenau Hospital	14,773.00	4/1/55
#63 - Simon, David L., University of Cincinnati Cincinnati General Hospital	2,800.00	4/15/55
#14 - Motley, Hurley Lee, University of Southern California	21,000.00	6/1/55
#51 - Pratt-Thomas, H. R., Medical College of South Carolina	8,134.50	7/1/55
#66 - Gruhzt, Carl C., University of Pennsylvania, Graduate School of Medicine	<u>13,915.00</u>	7/1/55
<u>TOTAL</u>	\$439,341.40	

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No.	Investigator	Institution	Subject	Amount	Date	Position
70	<u>FREUND</u> , Jack, M.D. Lecturer in Pharmacology of Virginia Assistant in Medicine	Medical College of Virginia	Correlation of multitechnical procedures performed on the peripheral circulation of normal individuals in recumbent and erect positions and after exercise before and after sham and actual smoking.	17,435.00	3/15	
71	<u>SPAIN</u> , David M., M.D. Pathologist	Waldemar Medical Research Foundation, Inc.	Study of host factors in experimental induction of pulmonary tumors in mice.	12,050.00	3/14	
72	<u>RIGDON</u> , R. H., M.D. Prof. of Pathology, Dir. of Laboratory of Experimental Research	University of Texas, Medical Branch	Study the effect of methylcholanthrene on the tissues of the duck. To compare the effect of methylcholanthrene on different tissue with emphasis on the reaction in the trachea when compared with the skin of the body and the web of the foot.	5,390.00	3/21	
73	<u>FARR</u> , Wanda K., Ph.D. Research Consultant	Private Consulting Laboratories	Analyses of tars and resins from impinged and settled cigarette smoke by means of microscopical, microchemical and electron microscopical techniques.	9,650.00	3/14	
74	<u>LEUCHTENBERGER</u> Cecillie Ph.D., Asso. Prof. of Cytology, School of Medicine	Western Reserve University, Institute of Pathology	Quantitative analysis of nucleoproteins in tissues from animals subjected to tobacco smoke by microspectrophotometry and interference microscopy correlated with cytological and histological studies.	23,199.24	3/14	
75	<u>WALKER</u> , Sheppard M. Associate Professor of Physiology	University of Louisville, School of Medicine	Effect of smoking and of blood levels of tobacco products on the heart.	10,220.00	4/1	
76	<u>HEALY</u> , Madeline E. McMahon, R. N.	Brusch Medical Center	Using the blood allergen diagnosis method of Healy to measure immunity and characteristics of 1000 individuals, smokers and non-smokers of varying ages, sexes, susceptibilities, habits, hobbies, temperaments, states of life, and occupations. This "Healy" test method study will furnish many clues to the problem of disease in general. It is a biochemical test, a truly scientific test, and the only one capable of measuring autonomic response.	121,460.00	4/8	

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<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
101	<u>ARMEN</u> , Robert N., M.D., Chief, Medical Service; <u>COHEN</u> , Sheldon, G. M.D., Attending in Allergy	Veterans Administra- tion Hospital, Wilkes-Barre, Pa.	The effect of forced inhalation of tobacco smoke on the cardiovascular system and pulmonary tissues of normal and tobacco sensitized rabbits.	5,250.00	6/17	
102	<u>CORNMAN</u> , Ivor, Ph.D., Head of Dept. of Cellular Physiology	Hazleton Labora- tories	The nature of effects of tobacco smoke acting directly upon tissues.	26,800.00	6/20	
103	<u>CANNATA</u> , Benjamin, V. M.D., Physician & Surgeon	Benlee Research Laboratories	1st Phase. The removal of tars and its derivatives from tobacco smoke. 2nd Phase. A complete study of the entire respiratory tract on animals who have been subjected to tobacco smoke with the tars and its deriva- tives removed.	595,000.00	6/23	
104	<u>BROZEK</u> , Josef, Ph.D., Associate Professor	University of Minnesota, School of Public Health, College of Medical Sciences	A quantitative study of biological characteristics of men associated with differences in their tendency to adopt and maintain different smok- ing habits.	15,000.00	6/27	
105	<u>DEICHMANN</u> , William B., Chairman and Professor of Pharmacology	University of Miami, School of Medicine	Determination of the effects of ab- sorption of tobacco extracts from the oral cavity of the mouse and dog on the incidence of cancer of the urinary bladder, and as influenced by diets containing a normal and an elevated content of fat.	20,636.75	7/8	
106	<u>PORDY</u> , Leon, M.D., Re- search Assistant in Cardiology	The Mount Sinai Hospital	Clinical evaluation of the cardio- vascular effects of tobacco smoking.	12,765.00	7/25	
107	<u>BLADES</u> , Brian, M.D., Professor of Surgery	The George Washing- ton University School of Medicine	The effects of stress on the produc- tion of lung cancer by direct ex- posure of lung tissue to high con- centrations of tobacco smoke.	16,440.00	7/25	
108	<u>WINSOR</u> , Travis, M.D.	Heart Research Foundation and The Hospital of the Good Samaritan	Effect of tobacco and/or its con- stituents upon the vascular system of man in health and in various disease states.	6,900.00	8/1	

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No.	Investigator	Institution	Subject	Amount	Date	Disposition
77	COOPER, Mervin and Dudley, Optometrists, Res. Dir. & Business Officer respectively	Glaucoma Research	To conduct research investigations to ascertain the merit of the theory (Copyright #A161437 - Class A) that habitual smoking of cigarettes is beneficial in the prevention and re- lief of simple chronic glaucoma.	91,000.00	4/15	T1R2
78	COLEMAN, Frank Philip, M.D., Asst. Prof. of Clinical Surgery	Medical College of Virginia	Effects of prolonged inhalation of cigarette smoke on the respiratory tract of sheep.	135,765.15	4/15	
79	KIRSNER, Joseph B., M.D.	The University of Chicago	Effect of tobacco smoking upon basal gastric secretions in man.	18,375.50	4/20	
80	FELLOWSHIP PROGRAM	50 institutions	Subjects to be chosen by the individual institutions.	25,000.00		
81	MC CARTHY, Francis P. M.D., Professor of Oral Medicine	Tufts University Dental School	Relationship between tobacco smoking and the incidence leuko- plakia of the oral cavity and the esophagus.	8,735.00	5/2	
82	SASLAW, Milton S., M.D., Director of Medical Research	National Children's Cardiac Hospital	Effects of smoking on beta hemo- lytic streptococci.	8,650.00	5/2	
83	SCHOUR, Isaac Dr.	University of Illinois, College of Dentistry	Effects of smoking on oral tissues of young adults. (Longitudinal and cross-sectional studies.)	18,597.69	5/5	
84	SCHOUR, Isaac Dr.	University of Illinois, College of Dentistry	Histologic changes in the oral, pharyngeal and nasal tissues of experimental animals subjected to tobacco smoke.	18,137.69	5/5	
85	SOROF, Sam, Ph.D. Research Associate	The Institute for Cancer Research & Lankenau Hospital Research Institute	Chemical and physical studies on the tissue proteins involved in chemical carcinogenesis.	14,423.00	5/11	

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No.	Investigator	Institution	Subject	Amount	Date	Disposition
86	<u>RITTENBERG</u> , Sydney C. Professor of Bacteriology	University of Southern California	Studies on the mechanism of bacterial metabolism of nicotine and related compounds. The ultimate goal of the project is the elucidation of the intermediary metabolism of nicotine oxidation.	4,104.00	5/12	
87	<u>BACHRACH</u> , William H., M.D. Chief, Gastroenterology Section, V.A. Hospital	University of Southern California School of Medicine	An investigation into the effects of smoking on gastrointestinal functions and symptoms in health and in digestive diseases.	10,600.00	5/16	
88	<u>KLASSEN</u> , Karl P., M.D. Prof. of Surgery; Chief, Div. of Thoracic Surgery	Ohio State University Medical Center	Influence of cigarettes upon the histology of the bronchial mucosa.	5,000.00	5/20	
89	<u>THOMAS</u> , Dr. Caroline Bedell, Asso. Prof. of Medicine	The Johns Hopkins School of Medicine	The significance of different individual patterns of circulatory response to cigarette smoking.	1,680.00	5/20	
90	<u>CRANSTON</u> , Hoy A. Director	Laboratory of Polarographic Analysis	Treatment of human neoplastic metastases with specific immune sera.	46,342.00	5/16	
91	<u>BING</u> , Richard J., M.D. Prof. of Experimental Medicine and Clinical Physiology	The Medical College of Alabama	The effect of smoking on the coronary blood flow and certain phases of myocardial metabolism in patients with arteriosclerotic or hypertensive cardiovascular disease.	11,110.00	5/24	
92	<u>TRAVELL</u> , Janet, M.D. Asso. Prof. of Clinical Pharmacology	Cornell University Medical College	Electrocardiographic effects of nicotine in the rabbit with experimental coronary atherosclerosis.	8,910.00	5/26	
93	<u>WECHSLER</u> , Richard L., M.D. Clinical Physiologist	Montefiore Hospital Institute of Research	Effect of cigarette smoking on cerebral blood flow, cerebral metabolism, blood gases, blood pH, arterial pulse pressure curves, electrocardiograms, and electroencephalograms.	10,000.00	5/28	

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No.	Investigator	Institution	Subject	Amount	Date	Disposition
29A	HOMBURGER, Freddie, M.D., Dir., Cancer Res. & Cancer Control Unit of Tufts at N. E. C. Hospital	New England Center Hospital (revision of 29 submitted through Tufts University)	A study on the effects of various components of tobacco and cigarette paper upon the behavior of trans- plantable tumors in various species including the behavior of human tumors transplanted into animal species.	28,990.00	6/3	
94	PATTERSON, John M., Assistant Prof; SMITH, Walter T., Asso. Prof.	Kentucky Research Foundation for U. of Kentucky	Fundamental approaches to the evalu- ation of tobacco in regard to quality and physiological properties.	30,883.52	5/29	
95	BUTT, E. M., M.D., Chief Pathologist, Prof. of Pathology at U. of So. Cal.	Los Angeles County Hospital (Univ. of Southern Californ- ia)	It is proposed to study the trace metal storage of pulmonary and liver tissue by spectrographic and chemi- cal methods. (see application)	13,687.44	6/8	
96	CAMERON, John A., Ph.D. Professor of Anatomy	Baylor University College of Dentis- try	Study of the effects of tobacco smoke and its component gases on living mammals.	3,500.00	6/9	
97	WOOD, J. Edwin, M.D. Instructor in Medicine at Boston University School of Medicine	Massachusetts Memo- rial Hospitals, Evans Memorial	The effect of <u>prolonged</u> inhalation of tobacco smoke and of <u>prolonged</u> ab- stinence from the use of tobacco on the peripheral vascular response to acute inhalation of tobacco smoke in man.	4,000.00	6/16	
98	HELLER, John H., M.D. Executive Director	New England In- stitute for Medi- cal Research	Investigation of the relationship of surface charge of inhaled particles on their retention in the lung.	15,525.00	6/20	
99	WACHTEL, Henry K., M.D. Scientific Director and President	Chemical Hormone Corporation	Investigations concerning the rela- tions existing between cancer disease and hormonal disturbances of the pituitary gland.	42,000.00	1/12	
100	STEINHAUS, Arthur H., Professor of Physi- ology	George Williams College	Does tobacco smoke influence the production or action of sex hormone. (see original application)	10,065.00	6/16	

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No.	Investigator	Institution	Subject	Amount	Date	Disposition
29B	HOMBURGER, F., M.D., Director	Tufts College Medical School, Dept. of Surgery	A study of the effects of cigarette tars upon the behavior of transplantable tumors in rodents employing a new technique of intrauterine tumor transplantation.	25,760.00	8/6	TIR Res. Bronte File
109	GOLDSCHMIEDT, Henry, Ph.D., Research Fellow	Sydenham Hospital, Dept. of Dentistry	Investigation of the saliva of smokers and non-smokers with regard to sodium thiocyanate content.	3,480.00	9/15	h
110	WILLIAMS, William L., M.D., Assistant Prof. of Pathology	Institute of Pathology, University of Tennessee	A proposed study of the effect of multiple respiratory infections on the production of cancer of the lung.	58,517.50	8/25	
4A	WENDER, Simon H., Research Professor of Chemistry	University of Oklahoma Research Institute	A qualitative and quantitative study of the individual polyphenol content of cigarette tobacco and of the smoke and "tars" resulting from cigarette smoking, and also to study the fate of these compounds in the animal respiratory system.	8,970.00	8/26	
111	PEARLMAN, Samuel J., M.D., Attending Surgeon	Michael Reese Hospital, Ear, Nose and Throat Dept.	To determine the normal by study of exfoliated cells in the area of the nose, throat and bronchi, in patients of all ages, and in both sexes. To transmit these findings to the ear, nose and throat specialist by way of his special journals. To make a limited study of older persons, primarily non-smokers and heavy smokers.	7,500.00	9/21	
112	ALPERT, Louis K., M.D., Clinical Professor of Medicine	The George Washington University	Observation of changes in normal human bronchial epithelial cells grown in tissue culture with prolonged exposure to extracts of cigarette smoke.	10,934.20	9/27	
113	PATTERSON, John L., M.D., Assoc. Professor of Medicine	Medical College of Virginia	Detection and prognostic significance of early obstructive pulmonary disease.	6,800.00	10/3	
114	SMITH, William E., M.D.	New York University-Bellevue Medical Center (now)	Experiments pertaining to lung tumors. 1. Exploration of specificity of a rapid test for carcinogenicity of chemicals. 2. Search for a virus in pulmonary adenomas of mice. 3. Investigation of dietary deficiency of choline in relation to lung tumors.	17,920.00	10/3	

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<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
115	THIENES, Clinton H., M.D., Ph.D., Director	Institute of Medical Research, Huntington Memorial Hospital	Effect of daily nicotine administra- tion upon adrenal glands, and upon mortality of the newborn.	6,925.00	10/10/55	B
116	KEYS, Ancel, Prof. & Director, Laboratory of Physiological Hygiene	University of Min- nesota, School of Public Health	Characteristics of men, including smoking, in populations differing in the incidence of coronary heart disease.	8,050.00	10/21/55	
117	RICHARDS, Victor, M.D., Prof. of Sur- gery	Stanford University School of Medicine	A comparative study of the effects of whole and fractionated extracts of cigarette smoke and those of known car- cinogens on I. The cytology and nuclear DNA content of epidermis in various strains of mice. and/or II. The cyto- logy and nuclear DNA content of lung and epithelium of the bronchial tree of mice and hamsters.	14,100.00	12/5/55	
118	HIGHTOWER, N. C., M.D., Ph.D.	Scott, Sherwood and Brindley Foundation for Medical Educa- tion and Research	Determination of the influence of smok- ing on gastric secretion and gastric motility in normal individuals and patients with duodenal ulcer.	2,369.00	12/5/55	
119	CHRISTENSEN, Kermit Professor of Anatomy	St. Louis Univer- sity School of Medi- cine	The relation of nicotine administra- tion to the secretion (or accumula- tion), identification, quantitation, and localization of noradrenalin and adrenalin compounds in the walls of blood vessels.	10,680.00	12/15/55	
120	RIGLER, Leo G. M.D.	City of Hope Medi- cal Center	A study of the development of carci- noma of the lung in smokers and non- smokers subjected to the influence of smog.	76,732.00	12/20/55	
121	YADOFF, Oleg, Presi- dent and Scientific Director	Foundation for Bio-Physico Re- search	Experimental research on the effect of tobacco, in particular nicotine, on tumor cells and cancer formation in small tropical Guppies, an aquarium- raised marine animal especially selected for this purpose.	12,100.00	1/3/56	

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<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
122	<u>LOWELL</u> , Francis C., M.D. Member, Evans Memorial Associate Prof. of Med. Boston U. Sch. of Med.	Evans Memorial, Massachusetts Memorial Hospitals	To determine in the course of the next 1½ or two years the incidence in an industrial population of chronic pulmonary disease (especially emphysema and asthma), and its relation to occupation, smoking and past history of certain diseases.	\$9,900	1/3	
123	<u>JACOBSON</u> , Jerry Hart, M.D., Director, Div. of Electrophysiology	New York Eye and Ear Infirmary	A comparison of electroretino- graphy as a means of evaluating the effect of vasoconstrictor drugs upon cerebral and retinal circulation to other techniques for this determination.	4,200	1/10	
124	<u>BOROTA</u> , Alexander, M.D., Assoc. Attending Dermatologist	Bird S. Color Memorial Hospital and Home	How smoking effects the oral mucosa of geriatric patients. a) Does smoking alone produce cancer or precancer of the oral mucosa? Yes or no. b) Does smoking alone predispose to cancer or precancer formation of the oral mucosa? Yes or no. c) The role and importance of other cancerogenic factors in the domestic and occupational environment?	\$19,032.50	3/7	
26A	<u>FULTON</u> , George P., Ph.D. Professor of Biology	Boston University Graduate School, Dept. of Biology	Direct observations on blood vessels during exposure to constituents of cigarette smoke.	\$14,720.00	3/12	
125	<u>ECKSTEIN</u> , John W., M.D. Assistant Prof. of Internal Medicine & Chief, Peripheral Vascular Laboratory, Cardiovascular Research Laboratories.	State University of Iowa, College of Medicine	Responses of the Peripheral Veins in Man to the Inhalation of Tobacco Smoke.	\$3,200.00	3/12	

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<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
126	<u>BONNER</u> , James, M.D.	California Institute of Technology	Enzymatic study of methylation reactions in plant tissues.	\$10,508.	3/20	
127	<u>TIECKE</u> , Richard W. D.D.S., Assoc. Prof. of Pathology	Northwestern Univ.	The Role of Tobacco in Oral Cancer.	8,573.	3/20	
128	<u>LARSON</u> , Paul S., Ph.D. Professor of Pharmacology	Medical College of Virginia	Enzymatic Transformations of Nicotine.	29,080.	3/20	
82A	<u>SASLAW</u> , Milton S., M.D. Director of Medical Research. <u>STREITFELD</u> , Murray M., Ph.D., Bacteriologist	National Children's Cardiac Hospital	Effects of Smoking on Beta Hemolytic Streptococci.	8,650.	5/2	
129	<u>KAISER</u> , E.R., Director of Research	American Society of Heating and Air Conditioning Engineers, Inc.	Tobacco Smoke Odor and Ventilation.	7,500.	4/4	
130	<u>HOLLAND</u> , Robert H., M.D. Chief, Thoracic Surgery	Veterans Administration Hospital	Effect of tobacco smoke on the respiratory passages of the rabbit.	17,500.	4/11	
131	<u>RIGDON</u> , R.H., M.D., Prof. of Pathology - Director, Laboratory of Experimental Pathology.	University of Texas Medical Branch	a) Compile all reference pertaining to cancer of the lung. b) Prepare paper on cancer of the lung between 1900 to 1930. c) Prepare paper on cancer of the lung between 1930 and 1935. d) Check cases of cancer of the lung so diagnosed in hospital with ultimate diagnosis as recorded on death certificates.	\$8,567.50	4/2	

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<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
132	OSTFELD, Adrian M., M.D. Assistant Prof. of Preventive Medicine	University of Illinois	A biomicroscopic assay of bulbar conjunctival vascular reactivity in hypertension employing topical and parenteral pharmacologic agents.	\$6,300.	4/19	
133	PERKINSON, Jesse D., Jr. M.D. Associate Prof. of Chemistry	The University of Tennessee	Relationship of tobacco leaf metabolism to degree of hydration.	\$11,730.	4/19	

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# 70	- Freund, Jack - Medical College of Virginia	\$17,435.00	10/15/55
# 95	- Butt, E. M., - Los Angeles County Hospital	13,687.44	1/ 1/56
# 4A	- Wender, Simon H. - University of Oklahoma, Research Institute	8,790.00	1/ 1/56
#116	- Keys, Ancel, University of Minnesota (Date of activation changed from January 1 to February 1, 1956)	8,050.00	2/ 1/56
#117	- Richards, Victor - Stanford University School of Medicine	33,800.00	3/ 1/56
#126	- Bonner, James - California Institute of Technology	9,680.00	9/ 1/56
#128	- Larson, Paul S. - Medical College of Virginia	29,080.00	7/ 1/56
#134(a)	- Tissue Culture Association	5,000.00	6/ 1/56
#134(b)	- Gey, George O. - Johns Hopkins University	8,000.00	7/ 1/56
#123	- Jacobson, Jerry Hart - New York Eye and Ear Infirmary	4,200.00	9/ 1/56
TOTAL		<u>\$802,939.97</u>	

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Factor 2

<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
140	<u>LOOMIS</u> , T.A., Ph.D., M.D. Associate Professor of Pharmacology. State Toxicologist.	University of Washington	Further studies of the broncho- constrictor factor contained in cigarette smoke.	\$8,935	10/2	
141	<u>KOSAK</u> , Alvin I., Ph.D. Associate Professor of Chemistry.	New York University	The isolation and identification of certain lower-boiling components of cigarette smoke.	\$6,038	10/11	
142	<u>SALTMAN</u> , Paul D., Ph.D. Assistant Professor Department of Biochemistry and Nutrition	University of Southern California	Some aspects of amino acid metabolism in tobacco leaves.	\$7,560	10/15	
143	<u>RIGDON</u> , R. H., M.D. Professor of Pathology Director, Laboratory of Experimental Pathology	University of Texas Medical Branch	Compile bibliography on cancer of the lung.	\$5,500	11/7	
144	<u>McDONALD</u> , Donald F., M.D. Associate Professor of Department of Surgery	University of Washington	Experimental carcinogenicity of tobacco smoke condensate extractives to transitional epithelium of the urinary bladder.	\$9,717	11/29	
145	<u>DOBROWOLSKI</u> , Tomasz B.	London, England	Investigation of comparative resistance to polio of tobacco users based on analysis of data concerning polio cases among adults and teenagers.	\$33,000	11/29	
146	<u>MONTGOMERY</u> , Hugh, M.D. Associate Professor of Medicine	Hospital of the University of Pennsylvania	Influence of nicotine (i.v.) and tobacco smoking on blood flow in human skin and skeletal muscle."	\$10,500	10/15	
147	<u>PROCTOR</u> , Richard C., M.D. Assistant Professor of Psychiatry and Neurology	Bowman Gray School of Medicine of Wake Forest College	An investigation of the emotional development of excessive smokers.	\$25,000	1/7/'57	

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<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
172	KNUDTSON, Kenneth P., M.D. Assistant Chief of Laboratory Service; Professor of Pathology	Veterans Administration Hospital and University of Washington	Histochemical study of changes in the buccal mucosa of mice following application of tobacco smoke condensate.	\$6,686.00	6/19	
173	LAIPPLY, Thomas C., M.D. Professor of Pathology, Northwestern Univ. Med. Sch., Chairman, Dept. of Labs., Chicago Wesley Memorial Hosp., WARTMAN, William B., M.D. Professor of Pathology and Chairman, Department of Pathology, Northwestern Univ. Med. Sch.	Northwestern University Medical School	Significance of atypical epithelial hyperplasia and car- cinoma in situ in relation of smoking, invasive bronchogenic carcinoma, and hormone therapy.	\$29,592.00	7/18	
174	HOLMAN, Russell L., M.D. Professor and Head of Dept., Department of Pathology	Louisiana State University	Relationship of smoking to acute myocardial infarction.	\$ 7,545.00	7/18	
175	SUNDERMAN, F. William, M.D., Ph.D., Director of the Division of Metabolic Research; Clinical Professor of Medicine	Jefferson Medical College	Metabolism of trace metals: role of metallic carbonyls in pulmonary carcino- genesis.	\$17,675.00	8/9	
176	HOMBURGER, Freddy, M.D. President, Bio-Research Laboratories, Inc. Scientific Associate, Roscoe B. Jackson Memorial Laboratory	Bio-Research Labora- tories, Inc.	Standardized quantita- tion of carcinogenic potency of selected carcinogens and development of bio- assays of rapid determination of carcinogenic potency of various substances.	\$70,038.00	7/13	

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<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
177	PAYZANT, Arthur R., M.D. Chief of the Radiology Department	Touro Infirmary	Determination of roentgeno- graphic changes in the plain EPA of the chest, if any, of vascular shadows as related to smoking of cigarettes, correla- ted to length of time of smok- ing and type of smoking habits.	\$5,000.00	8/8	
178	DEAN, Geoffrey Kerfoot, M.B., Ch.B., M.D., M.R. C.P., Consulting Physician, Senior Honor- ary Physician	Eastern Cape Provincial Hospital	(1) To investigate the true incidence of carcinoma of the bronchus in the 3,000,000 white South Africans. (2) To investi- gate the environment of the comparatively small number of South Africans who have died of lung cancer in the last five years. (3) To assess the importance of other environmental factors than smoking in the causation of lung cancer.	\$30,000.00	10/15	
179	HAKIM, Anwar A., Ph.D. Enzyme Biochemist, Department of Medical Research	National Children's Cardiac Hospital	Isolation and identification of abnormal constituents in the blood stream, induced by smoking.	\$14,650.00	10/18	
180	FREEDLANDER, B.L., M.D. Director of Cancer Research	Mount Zion Hospital	Experiment on the carcinogenic and cocarcinogenic action of tobacco products.	\$16,125.00	10/18	
181	RUBIN, Benjamin A., Ph.D. Assistant Professor, Department of Public Health and Preventive Medicine	Baylor University College of Medicine	An evaluation of the phenom- enon of tumor growth enhance- ment as an assay for carcino- gens among the polycyclic hydrocarbons and related compounds.	\$ 5,175.00	1/6/58	
182	SKORYNA, Stanley C., M.D. Research Director, Department of Experimental Surgery	McGill University	Effects of application of tobacco tars on buccal mucosa.	\$ 9,800.00	9/3/57	

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<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
183	DERRICK, John R., M.D. Assistant Professor of Surgery, Head of Section of Cardiovascular Surgery	University of Texas Medical Branch	Smoke and air dynamics of the tracheobronchial tree (determining methods of study).	\$8,800.00	9/17/57	
184	MAKARI, Jack G., M.D., Sc.M., D.T.M.&H. Director of Research	Muhlenberg Hospital	Studies on cigarette smoke condensates by immunologic methods. (I) Basic immuno- logic studies (II) Immuno- epidemiologic studies (III) Immuno-pathologic studies.	\$46,000.00	10/23/57	
185	FALK, Hans L., Ph.D. Visiting Associate Professor of Biochemistry and Nutrition	University of Southern California	A compilation of fluorescence spectra of polycyclic aromatic hydrocarbons and closely related compounds, which are of interest in the study of air pollutants, and cigarette smoke in relation to lung cancer etiology.	\$7,475.00	10/28/57	
186	RIKER, Walter F., Jr., M.D. Professor of Pharmacology	New York State Society for Medical Research	Support for New York State Society for Medical Research.	\$5,000.00	12/6/57	
187	SILVETTE, Herbert, B.S., M.S., Ph.D., Visiting Professor Pharmacology	Medical College of Virginia	Immunological investigation of tobacco and tobacco smoke.	\$16,225.00	12/13/57	
188	WELLS, Roe E., Jr., M.D. Associate in Medicine	Peter Bent Brigham Hospital	A study of the correlation of smoking history and habits with pulmonary function and the acute effects of smoking thereon in patients with bronchitis, bronchial asthma, and the common cold with similar studies in a selected geriatric population of apparent good health.	\$17,325.00	1/3/58	

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<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
189	HUANG, T.C., Ph.D. Director of Research	Timken Mercy Hospital	To study the soluble and the non-soluble substances from tobacco smoke upon the following systems of the body: 1. Reproductive system and growth. 2. Respiratory system.	\$,440.00	1/9/58	
190	KEYS, Ancel, Ph.D. Professor, School of Public Health, and Director, Laboratory of Physiological Hygiene	University of Minnesota	It is proposed to study inter-relations in men in different populations between prevalence and incidence of hypertensive and coronary heart disease, smoking habit and other characteristics of possible relevance (e.g., occupation, dietary custom, relative obesity, blood lipids, skeletal type).	\$20,504.00	1/16/58	
191	LAWTON, M. Powell, Ph.D. Assistant Director Psychological Services	Norristown State Hospital	A prospective and retrospective study of the psychophysiological correlates of smoking.	\$18,782.00	1/22/58	

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No.	Investigator	Institution	Subject	Amount	Date	Disposition
189	HUANG, T.C., Ph.D. Director of Research	Timken Mercy Hospital	To study the soluble and the non-soluble substances from tobacco smoke upon the following systems of the body: 1. Reproductive system and growth. 2. Respiratory system.	\$ 440.00	1/9/58	
190	KEYS, Ancel, Ph.D. Professor, School of Public Health, and Director, Laboratory of Physiological Hygiene	University of Minnesota	It is proposed to study inter-relations in men in different populations between prevalence and incidence of hypertensive and coronary heart disease, smoking habit and other characteristics of possible relevance (e.g., occupation, dietary custom, relative obesity, blood lipids, skeletal type).	\$20,504.00	1/16/58	
191	LAWTON, M. Powell, Ph.D. Assistant Director Psychological Services	Norristown State Hospital	A prospective and retrospective study of the psychophysiological correlates of smoking.	\$18,782.00	1/22/58	
192	CATTELL, Raymond B., Ph.D. Senior Director Research Professor in Psychology	University of Illinois	A twelve year study of personality and personality changes in relation to smoking behavior.	\$23,300.00	2/6/58	
193	GERSHBEIN, Leon Lee, Ph.D. Adjunct Associate Professor of Biochemistry	Illinois Institute of Technology	Derivation of a possible rapid method for the screening of carcinogens based on the extent of liver regeneration in partially hepatectomized animals; application of the procedures to various hydrocarbons, tobacco tar fractions and smoke.	\$10,570.00	1/17/58	

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<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
194	PACE, Donald M., Ph.D. Professor & Chairman, Department of Physiology and Director, Institute for Cellular Research	University of Nebraska	To study the effects of certain constituents of tobacco smoke on tissue cells cultivated in vitro.	\$ 8,000.00	1/28/58	
195	ARMSTRONG, Bruce W., M.D. Director, Cardio-Pulmonary Laboratory	University Hospital	Cardio-pulmonary function and exercise tolerance in smokers and non-smokers.	\$17,326.00	2/1/58	
196	GOLDMAN, Leon, M.D. Professor of Dermatology	Cincinnati General Hospital	Study of the irritant effect of identified tobacco smoke tars on normal and pathologic human skin and mucous membrane.	\$10,600.00	3/3/58	
197	BYERRUM, Richard U., Ph.D. Professor of Chemistry	Michigan State University	Biosynthesis of the pyridine ring of nicotine.	\$ 6,984.00	2/26/58	
198	SEGAL, Maurice S., M.D. Clinical Professor of Medicine	Tufts University School of Medicine	Relationship of cigarette smok- ing to chronic (obstructive) pulmonary emphysema.	\$ 5,000.00	3/20/58	
199	HARRIS, Irving D., M.D. Psychiatric Research Consultant	Child Guidance Research Fund, Inc.	Personality factors in cigarette smoking. The aim of the pro- posed work is to investigate what personality and emotional factors are associated with excessive smoking of cigarettes and with an increase in the rate of cigarette smoking.	\$10,300.00	3/27/58	
200	COOPER, Philip, M.D. Chief, Surgical Service Clinical Professor of Surgery	Veterans Administration Hospital	A study of the effect of extracts of tobacco on cultures of tumor and normal cells. Animal trans- plants of tumor tissue from tissue cultures.	\$20,575.00	4/2/58	
201	BOWERY, T.G., Ph.D. Pesticide Residue Laboratory Chemistry Department	North Carolina Agricultural Experiment Station	Site and level of deposition of TDE and endrin residue components of cigarette smoke in experimental animals.	\$18,548.00	4/7/58	

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<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
202	RABB, R. L. Acting Director of Project	North Carolina State College	Insect pathogens of tobacco insects.	\$12,200.00	4/4/58	
203	THOMAS, Caroline B., M.D. Associate Professor of Medicine	The Johns Hopkins School of Medicine	(a) Studies of genetic differ- ences between smokers and non- smokers. (b) Studies of psycho- logical differences between smokers and nonsmokers as shown by comparison of figure drawings.	\$11,500.00	4/15/58	
204	CONNORS, Dean M., M.D. Pathologist & Medical Director	A. D. Daniels Memorial Laboratory of St. Mary's Hospital	A study of the alterations in the human bronchial wall occur- ring with aging, with particular emphasis on elastic tissue changes and associated changes in the bronchial lumen size.	\$ 5,000.00	4/29/58	
205	TAVARES, Clement A., M.D.	453 Blackstone Avenue Fresno, California	The energy concept of cancer causation.	\$ 1,500.00	6/10/58	
206	DOMINO, Edward F., M.D. Assistant Professor of Pharmacology	University of Michigan	Effects of tobacco smoke and nicotine on the central nervous system.	\$11,500.00	6/16/58	
207	REICH, Carl J.	615 - 6 Street New Westminster, B.C.	Synergistic vitamin D and calcium deficiency a new and variable syndrome in the adult.	\$5-6000.00	5/14/58	

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No.	Investigator	Institution	Subject	Amount	Date	Disposition
220	MILLER, James G., M.D. Ph.D., Professor of Psychiatry and Psychol- ogy, and Director, Mental Health Research Institute	University of Michigan	The behavioral effects of smoking under stress.	\$10,000.00	11/29/58	
221	BELLET, Samuel, M.D. Director, Division of Cardiology	Philadelphia General Hospital	1. The effect of nicotine on the fat handling: comparison of a group of smokers and non- smokers. 2. The effect of nicotine on cardiac irritability in the presence of reserpine. 3. The effect of nicotine on coronary blood flow of dogs with coronary insufficiency.	\$ 8,970.00	11/21/58	
222	BOOK, Fred G., Ph.D. Senior Cancer Research Scientist	Health Research, Inc. Roswell Park Division	Investigation of the carcino- genic effect of medicinal coal tar preparations.	\$17,250.00	11/28/58	
223	LARSON, Paul S., Ph.D. Professor of Pharmacology	Medical College of Virginia	Enzymatic transformations of nicotine and related compounds.	\$33,033.00	12/7/58	
224	HOMBURGER, Freddy, M.D. President and Director	Bio-Research Institute, Inc.	Studies of carcinogenic properties of various tobacco condensates in skin of mice.	\$28,750.00	12/23/58	
225	McKENNIS, Herbert, Jr. Ph.D., Professor of Pharmacology	Medical College of Virginia	Metabolism of antinicotinic acid compounds of tobacco smoke.	\$21,329.00	12/19/58	
226	CERECEDO, Leopold R. Ph.D., Professor of Biochemistry	Fordham University	A study of early chemical changes in the lungs of tumor- bearing rats and mice.	\$13,200.00	2/11/59	
227	RIGDON, R.H., M.D. Professor of Pathology	The University of Texas Medical Branch	Bibliography - cancer of the lung - 1810 to 1958.	\$ 7,500.00	2/4/59	
228	KEYS, Ancel, Ph.D. Professor, School of Public Health; Director, Laboratory of Physiolog- ical Hygiene	University of Minnesota	Characteristics of smokers and non-smokers in different populations.	\$ 9,775.00	2/4/59	

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<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
229	LEUCHTENBERGER, Cecilie, Ph.D., Senior Biologist and Cytochemist	Children's Cancer Research Foundation	A correlated histological, cytological and cytochemical study of the tracheo-bronchial tree from mice exposed to cigarette smoke.	\$13,000.00	2/9/59	

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APPROVED GRANTS

1003537472

January 18, 1954

MEMORANDUM TO: All Members
Tobacco Industry Research Committee

Attached is a list of Cancer Research grants, a composite breakdown assembled from American Cancer Society reports. This was prepared at the request of the Company Research Directors' Advisory Committee.

Descriptions under the heading "Research Studies" are, of necessity abbreviated. For more complete information on each individual grant the ACS or the individual institution should be consulted.

We are gathering additional data to keep this report up to date.

Hill and Knowlton, Inc.

1003537473

October 1, 1953

COMPOSITE LIST OF CURRENTLY OPERATING
FELLOWSHIPS, GRANTS-IN-AID, AND INSTITUTIONAL RESEARCH GRANTS
TO CANCER RESEARCH FROM MAJOR AGENCIES
ACTIVE ON OCTOBER 1, 1953

American Cancer Society	ACS
Atomic Energy Commission	AEC - Government
Damon Runyon Memorial Fund	DRMF
Elsa U. Pardee Foundation	EUPF
Jane Coffin Childs Memorial Fund	JCC
National Cancer Institute	NCI - Government

(Prepared from data available on October 1, 1953)
(Parentheses following name of investigator gives code number
of grant for file purposes)

	Amount	Agency	Research Subjects
<u>ALABAMA</u>			
1. <u>Alabama Polytechnic Institute</u> (Auburn)			
Salmon (N-1F)	\$ 10,000	ACS	Nutrition
Salmon (C-1018 G4)	18,800	NCI	
Salmon (C-1018 C5)	18,800	NCI	
	47,600		
2. <u>Medical College of Alabama</u> (Birmingham)			
Whiteside-Carlson & Carlson (C-1901)	6,480	NCI	Lipides, cell division
Carlson & Whiteside-Carlson (C-1902)	6,696	NCI	Tumor-inhibitory alkylating agents
	13,176		
3. <u>Southern Research Institute</u> (Birmingham)			
Murray (DMSTR-38E)	52,909	ACS	Useful chemotherapeutic agents
Skipper (BI-5F)	20,000	ACS	Anti-cancer & radioactive anti-leukemic agents
Skipper (C-1184 G2)	8,564	NCI	
	81,473		
4. <u>Tuskegee Institute</u> (Tuskegee Institute)			
Henderson (CP-51A)	3,500	ACS	Sunflower callus tissue
Henderson (C-1632 G)	2,500	NCI	
	6,000		
<u>ARKANSAS</u>			
<u>University of Arkansas</u> (Little Rock & Fayetteville)			
Dinning (GFB-20B)	6,912	ACS	Leukemia, metabolism nucleic acids
Johnson (MOR-23)	5,000	ACS	Growth & morphology bacteria
Meschan (C-1866)	9,277	NCI	Isodose curves
Nettleship (ENV-1B)	5,000	ACS	Neoplastic growth, trauma, skin transplants
	26,189		
<u>CALIFORNIA</u>			
1. <u>California Institute of Technology</u> (Pasadena)			
Borsook (FR-17B)	7,500	ACS	Proteins, isotopes
Galston (CP-50A)	4,500	ACS	Catalase activity, plants
Niemann et al. (C-354 G4)	13,000	NCI	Tumor hemorrhagic agent
	25,000		

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	Amount	Agency	Research Subjects
2. <u>University of California</u> (Berkeley, Los Angeles & San Francisco)			
Adelberg (MET-13A)	4,000	ACS	Bacterial mutants, sterols
Bassett (CIE-6F)	Ext. time	ACS	Steroid hormones, metabolism
Berg (MOR-14C)	Ext. time	ACS	Mytilus edulis
Chaikoff (IS-6F)	8,000	ACS	Radioactive isotopes, cholesterol metabolism
Chaikoff (C-879 C4)	24,823	NCI	
Cohen (F-120B)	Fellow	ACS	Morphogenesis tobacco leaves
Eakin & Berg (C-1554 C2)	2,754	NCI	Protein metabolism, amphibian embryo
Eiler (G-448 C4)	6,843	NCI	Electro-enzyme chemistry
Flickinger (MOR-20)	3,500	ACS	Isotopes, amphibian embryo, C ¹⁴ O ₂
Greenberg (MET-12B)	6,000	ACS	Isotopes, amino acids
Greenberg (DRIR-206A)	13,920	DRMF	
Greenberg (C-327 C5)	8,500	NCI	
Griffith (C-1669 C)	10,000	NCI	Beta-aminoisobutyric acid
Hinton (CP-17E)	8,000	ACS	Chromosomes, diet, Drosophila
Kirk (C-403 C5s)	300	NCI	Cytological chemistry,
Kirk (C-403 C6)	14,526	NCI	Tissue culture
Lawrence & Berlin (C-1440 C2)	13,679	NCI	Red blood cells
Li (H-16A)	15,000	ACS	Hypophyseal hormone
Madden & Zeldis (C-1983)	19,667	NCI	Protein, cells, nitrogen equilibrium
Mazia (E-9G)	7,547	ACS	Enzyme chemistry, chromosomes
Roberts (C-1408 C2)	17,863	NCI	Serum proteins
Simpson (C-1098 C3)	10,000	NCI	Growth hormone
Stanier (E-29D)	8,100	ACS	Enzymes
Stumpf (MET-4C)	7,765	ACS	Metabolism, plants
Tarver (MET-16A)	7,500	ACS	Protein metabolism, isotopes
Williams (PH-3G)	8,964	ACS	Protein macromolecules
Wood (INSTR-43E)	75,000	ACS	Follow-up, cases
	302,251		
3. <u>Cedars of Lebanon Hospital</u> (Los Angeles)			
Friedman (C-1789)	5,751	NCI	Masculinizing ovarian tumors
Henstell (BCH-5A)	5,000	ACS	Desoxyribonuclease
	10,751		
4. <u>Donner Laboratory of Medical Physics</u> (Berkeley)			
Maisin (DRF-127A)	Fellow	DRMF	Radioiron
5. <u>Laboratory for Research on Treatment of Cancer</u> (Boulder Creek)			
Turner (C-957 C5)	4,900	NCI	Treatment
6. <u>Los Angeles County Hospital</u> (Los Angeles)			
Pearson (C-1830)	4,487	NCI	Frogs
Pearson & Visser (C-962 C3)	12,500	NCI	Theiler's virus
	16,987		
7. <u>Mt. Zion Hospital</u> (San Francisco)			
Freedlander (CH-6A)	7,400	ACS	Chemotherapy
Freedlander (DRIR-93B)	7,400	DRMF	
	14,800		
8. <u>Palo Alto Foundation</u> (Palo Alto)			
Salzberg (SG-14)	Scholar	ACS	Azo dye
9. <u>University of Redlands</u> (Redlands)			
Baez (PH-17)	3,700	ACS	X-ray
10. <u>Rees Stealy Clinical Research Foundation</u> (San Diego)			
Stimmel (EDC-10)	5,000	ACS	Excreta, estrogens
Stimmel (C-759 C5)	5,000	NCI	
	10,000		

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11. Santa Barbara Botanic Garden (Santa Barbara)
Walters (C-1513 C2)
12. University of Southern California (Los Angeles)
Edmondson (C-1904)
Lobstein
Macdonald (INSTR-66A)
Macdonald & Guiss (C-1862)
Rittenberg (N-19A)
Starr (C-1214 C2)
Starr et al. (C-1868)

13. Stanford University (Stanford)
Cutting (DRIR-201A)
Danforth (C-1358 C2)
Giese (C-1799)
Ginzton & Kaplan (INSP-65A)
Griffin (PR-15C)
Griffin (DRIR-237)
Griffin (C-1145 C3)
Kaplan (#107)
Kirkman (EDC-5)
Kirkman (C-1579 C)
Kirkpatrick (PH-15A)
Loring (PR-12D)
Luck (BCH-8A)
Luck (C-484 C5)
Tatum (#86)
Tatum & Barratt (EG-27E)
Twitty (CP-13F)
Waxler (C-1518 C)

Amount	Agency	Research Projects
6,370	NCI	Chromosomes, Bromus hybrids
4,428	NCI	Estrogens, liver
2,000	EUPF	Related fields
24,109	ACS	Research coordinating plan
6,653	NCI	
2,376	ACS	Oxidative assimilation
14,310	NCI	Thyrotrophic hormone,
9,342	NCI	Thyroxin
63,218		
9,700	DRMF	Chemotherapy
7,236	NCI	Mutagenic effects nitrogen mustards
4,675	NCI	Ultraviolet radiation
24,915	ACS	Radiation therapy
4,698	ACS	Nucleoproteins
8,035	DRMF	Pituitary factors
4,914	NCI	Nitrogen mustards
12,800	JCC	Lymphoid tumor
5,184	ACS	Hormone induced fibromas
3,000	NCI	
9,000	ACS	X-ray microscopy
8,500	ACS	Nucleic acid
5,000	ACS	Histones, proteins
24,946	NCI	
5,000	JCC	Induced mutations
4,860	ACS	Neurospora
8,000	ACS	Pigment cells
7,668	NCI	Obesity, gold thioglucose
158,131		

COLORADO

- University of Colorado (Denver)
Darley (INSTR-55C)
Darley (DRIR-155B)
Fink (CP-39C)
Herrmann (#102)
Scharrer (CP-1F)

25,000	ACS	Cellular biology,
7,800	DRMF	endocrinology
3,024	ACS	Tissue autoantigens & autoantibodies
6,500	JCC	Radioactive tracers, muscle tissue
8,640	ACS	Neuro-endocrine factors
50,964		

CONNECTICUT

1. Connecticut College (New London)
Christiansen (CP-48A)
2. University of Connecticut (Storrs)
Friedland (PH-14A)
Kind (BCH-14)
Landauer (EG-7F)
3. Wesleyan University (Middletown)
Cochrane (MET-18)

6,000	ACS	Cell metabolism, growth, division
Ext. time	ACS	Mass spectrometer & steroids
4,000	ACS	Phosphoprotein phosphatase
4,500	ACS	Physiology of phenocopies
8,500		
2,000	ACS	Pentose metabolism in microorganisms

1003537476

5. Yale University (New Haven)

Albrink (MOR-21)
 Banfield et al. (C-1886)
 Bonner (BO-12H)
 Bunting (C-383 C5)
 Busch (#110)
 Duran-Reynals (V-8G)
 Duran-Reynals (#20)
 Duran-Reynals (C-997 C4)
 Fruton (E-14G)
 Gardner (#13)
 Gaylord (#112)
 Greene (CP-11F) (S in n)
 Greene (#43) (1A)
 Greene (C-918 C4)
 Hakala (#61-33)
 Lippard (INSTR-47E)
 Nichol (SG-8)
 Nicholas & Boell (MOR-9E)
 Parfentjev (C-1201 C3)
 Setlow (PH-20)
 Sokal (V-5)
 Srere (#61-31)
 Strong (EG-38A)
 Strong (C-932 C4)
 Trentin (#97)
 Trentin (C-1491 C2)
 Van Eck (C-1656 C)
 Van Eck (C-1656 Cs)
 Vogel (DRF-109A)
 Wagner (C-1998)
 Welch (CB-7A)
 Welch (DRIB-248)
 Winternitz (#113)

Amount

Agency

Research Subjects

6,000	ACS	Animal & <u>in vitro</u> transplantation
28,091	NCI	Electron microscopy of tissues
5,400	ACS	Genetic control, enzyme specificity
10,098	NCI	Histochemistry
9,000	JCC	Metabolism, <u>in vivo</u>
10,000	ACS	Virus infection
16,500	JCC	
18,306	NCI	
9,400	ACS	Peptide bonds, proteinases
6,800	JCC	Hormonal imbalances
5,000	JCC	Virus development
12,500	ACS	Tissues
25,464	JCC	
12,000	NCI	
3,800	JCC	Nucleic acids
50,000	ACS	Experimental & clinical
Scholar	ACS	Folic acid
9,000	ACS	Embryos
13,524	NCI	Immunochemical differences in sera
8,251	ACS	Spectra biological compounds
6,000	ACS	Heterologous transplants
4,000	JCC	Cholesterol in liver
5,000	ACS	Methylcholanthrene,
10,303	NCI	Gastric lesions
2,000	JCC	Mammary gland,
3,872	NCI	Lactation
9,000	NCI	X-ray, ovary
3,500	NCI	
Fellow	DRMF	Enzymatic adaptation
8,000	NCI	Virus multiplication
28,000	ACS	Pyrimidines
10,750	DRMF	
12,000	JCC	Metabolism, transplants
361,559		

DELAWARE

University of Delaware (Newark)
 Feeny (INSTR-34D)

Ext. time

ACS

Cathode rays,
 living cells

DISTRICT OF COLUMBIA

1. George Washington University

Corman (C-1234 C2)
 Corman (C-1939)
 Klopp (INSTR-24F)
 Kok (BAF-23)
 Smith (C-308 C6)
 Smith & Alpert (DRIB-42C)

3,793
 5,377
 25,000
 Fellow
 10,584
 10,000
 54,754

NCI
 NCI
 ACS
 ACS
 NCI
 DRMF

Proliferation, antibiotics
 Clinical & research
 Reticulosis
 Control substances
 Radioisotopes

100353247

	Amount	Agency	Research Subjects
2. <u>Georgetown University</u> Sullivan (C-1340 C3)	4,887	NCI	Tryptophan-kynurenine metabolism
3. <u>Howard University</u> Marshall (C-1420 C2)	4,000	NCI	Krebs tricarboxylic acid cycle
McKinney (C-1676 C)	7,000	NCI	Tissue cultures
Newman (DRIR-111B)	5,400	DRMF	Amino acids
Newman & Marshall (C-1874 R)	11,674	NCI	Embryogenesis, tricarboxylic acid cycle
	28,074		
4. <u>National Academy of Sciences</u> Committee on Growth (INST-12H)	108,756	ACS	Operating budget
Grady (INST-64)	15,000	ACS	Preparation educational material
Heumann (CBC-1H)	27,000	NCI	Chemical-biological correlation
Heumann (C-366 C5)	14,175	ACS	
Lucke (INST-27E)	1,000	JCC	Asst. Atlas of Tumor
Lucke (#96)	28,900	ACS	Pathology
Wason (INSP-75)	1,000	ACS	Budget, Comm. C. Diagnosis & Therapy
Weiss (INST-12A)	2,500	DRMF	Comm. Animal resources
Winternitz (DRIR-80B)	203,581		Diagnosis & therapy

FLORIDA

1. <u>Dade County Cancer Institute</u> (Miami) Grand (DRIR-216A)	7,800	DRMF	Antibiotics & chemotherapeutic agents
Hopman (C-1703 C)	9,936	NCI	Biopsy cytology
	17,736		
2. <u>Florida Southern College</u> (Lakeland) Sokoloff (DRIR-156B)	2,800	DRMF	Ascorbic acid
3. <u>Florida State University</u> (Tallahassee) Metz (GP-44B)	3,888	ACS	Fertilization
4. <u>University of Florida</u> (Gainesville) Ray (CH-14)	11,550	ACS	Therapeutic compounds
Ray (DRIR-33C)	17,500	DRMF	Radioactive derivatives
Ray (C-1066 C3)	12,000	NCI	Acetylaminofluorene
Ray (C-1308 C2)	5,000	NCI	Radioactive sulfur compounds
Ray (C-1356 C2)	7,900	NCI	
	53,950		
5. <u>University of Miami</u> (Coral Gables) Dunning (GP-6F)	Ext. time	ACS	Cysticercus fasciolaris
Dunning (N-15A)	4,000	ACS	Nucleic acid diet
Dunning (C-1861)	7,020	NCI	Immunity
Dunning (C-1864)	2,700	NCI	
Paff (C-2007)	9,000	NCI	Living mast cell
	22,900		

GEORGIA

1. <u>Emory University</u> (Emory University) Foraker (C-1486 C2)	7,000	NCI	Exfoliative cytology
Russell & Wilhelm (EEP-6G)	6,750	ACS	Nitrogen
	13,750		
2. <u>Oglethorpe University</u> (Oglethorpe) Cohen (CP-36C)	3,670	ACS	Protoplasmic organization

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IDAHO

University of Idaho (Moscow)
Beck (C-1802)

Amount

6,284

Agency

NCI

Research Subjects

Clostridium acidu urici

ILLINOIS

1. Cancer Research Inc. (Chicago)
"Cancer Research" (#36)

3,000

JCC

Support, Jour. Cancer Research

2. Carle Foundation (Urbana)
Quastler (C-302 C5)

6,480

NCI

Med. application, betatron

3. Chicago Medical School (Chicago)
Davidsohn (C-1113 C3)
Elias & Popper (C-1961)
Slubik (DRIR-261)

14,148

NCI

Antibodies

5,810

NCI

Liver

4,860

DRMF

Cortisone

24,818

4. University of Chicago (Chicago)

Coggeshall (INST-14F)

165,000

ACS

Adrenalectomy patients, antigens,

Coggeshall (DRIR-234)

28,000

DRMF

Radioisotopes

DeBruyn (R-9D)

6,588

ACS

Selective radiation

Doyle (E-26D)

5,076

ACS

Enzymatic histochemistry

Geiling (C-1652 C)

5,562

NCI

Radioactive colchicine

Harary (F-143A)

Fellow

ACS

Amino acids

Huggins (CBC-3E)

55,000

ACS

Chemotherapy

Huggins (DRIR-2C)

12,000

DRMF

Hormonal control

Huggins (#105)

24,700

JCC

Steroid hormones, pituitary principles

Kenyon et al. (CIE-2G)

9,871

ACS

Tissue & leukemia & ACTH

Pierce (CFB-21A)

4,000

ACS

and/or aminopterin

Pierce (C-1300 C4)

7,500

NCI

Proteins, multiple myeloma

Putnam (C-1331 C2)

7,080

NCI

Brain & beta ray

Rasmussen (C-1565 C)

8,000

NCI

Gastrointestinal tract

Rubin (DRF-102s)

Fellow

DRMF

Proteins & nucleic acids

Swift (C-1612 C)

2,500

NCI

Triphosphopyridine nucleotide

Vennesland (E-34C)

7,500

ACS

Morphogenesis

Weiss (MOR-10E)

20,000

ACS

Metabolism, sodium & potassium

Williams-Ashman (SG-10)

Scholar

ACS

tissue content

367,747

5. Illinois Institute of Technology (Chicago)
Danforth (MET-19)

1,500

ACS

Oxidative metabolism in Euglena

6. University of Illinois (Chicago)

Black (BO-1F)

14,250

ACS

Plant tumors & virus

Burstone (C-1373 C2)

5,600

NCI

Oral epithelium & radioactivity

Catchpole (KEP-12D)

6,000

ACS

Reproductive organs & hormones

Grant (C-1110 C3)

3,800

NCI

Gastric mucosa

Harvey & Bennett (C-854 C3)

7,452

NCI

Betatron electron & bone, cartilage

Kirschbaum (BI-10D)

4,725

ACS

Leukemia therapy

Kirschbaum (C-1543 Cs)

406

NCI

Adrenal cortical secretion

Kirschbaum (C-1969)

9,936

NCI

Mammary glands

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	Amount	Agency	Research Projects
<u>University of Illinois (Cont'd)</u>			
Kummerow (CH-12)	2,000	ACS	P-dimethylaminoazobenzene
Luria (V-3G)	24,450	ACS	Virus growth & structure
Nance (CP-43B)	3,780	ACS	Indole-3-acetic acid & pyruvate
Odell (CS-15A)	Ext. time	ACS	Gynecologic cancer
Plummer (C-1986)	6,804	NCI	Tissue cultures & amelanotic melanomas
Spiegelman (C-1094 C3)	9,900	NCI	Intracellular enzymes
Vestling (C-1856)	9,965	NCI	Liver enzymes
Winzler (PR-14C)	Ext. time	ACS	Plasma mucoprotein
Winzler (C-1828)	12,000	NCI	Amino acids
	121,068		
7. <u>Loyola University</u> (Chicago)			
Melchior (H-9B)	5,000	ACS	Enzyme systems in pituitary gland
8. <u>Michael Reese Hospital</u> (Chicago)			
Schwarz (DRF-120A)	Fellow	DRMF	Metastasis urinary bladder, prostate
Tannenbaum (DRIR-250)	13,500	DRIR	Nutrition
Tannenbaum (C-248 C7)	12,000	NCI	
	25,500		
9. <u>Northwestern University</u> (Chicago & Evanston)			
Preston & Schrek (VI-4)	1,800	ACS	Immunity to transplants
Wartman (C-1005 C3)	15,000	NCI	Pathological lesions
Fogelson	1,000	EUPF	Enzymes & related chemicals
10. <u>Southern Illinois University</u> (Carbondale)			
Lindgren (C-1179 C3)	17,800	NCI	Enzymes, yeasts
Lindgren & Sheffner (N-17A)	5,000	ACS	Amino acids & polypeptides & proteins
	8,000		
	13,000		
<u>INDIANA</u>			
1. <u>Indiana University Foundation Research Division</u> (Bloomington)			
Campaigne (C-1948)	8,240	NCI	Naphthobenzthiophene series
Haurowitz (PR-19B)	6,000	ACS	Protein-protein interaction
Lawrence (CS-11B)	Ext. time	ACS	Venous pathways, bone, transplants
Muller (EG-9G)	12,000	ACS	Mutations in Drosophila
Muller (C-382 C5)	9,500	NCI	Lymphomas & leukemia
Smith (C-1602 C)	7,961	NCI	Heredity in Paramecium
Sonneborn (EG-31C)	8,187	ACS	
	51,888		
2. <u>University of Notre Dame</u> (Notre Dame)			
Campbell (CH-3C)	2,500	ACS	Unsaturated lactones
Reyniers (DRIR-48C)	20,500	DRMF	Germ-free life
	23,000		
3. <u>Purdue University</u> (Lafayette)			
Benzner (VI-1)	4,300	ACS	Bacteria, ultraviolet radiation
Garner (SG-3)	Scholar	ACS	Microorganisms, mutagenesis
	4,300		
<u>IOWA</u>			
1. <u>Iowa State College</u> (Ames)			
Dahn (CP-49A)	3,600	ACS	Autoradiographic study, insects
Sinsheimer (PH-19)	2,570	ACS	Nucleotides
	6,170		

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2. State University of Iowa (Iowa City)

Nelson (INSTR-77)	10,000
Winnick (CP-60)	4,000
Winnick (C-1765)	6,480
Witschi (MOR-11E)	Ext. time
	26,650

KANSAS

1. Kansas State College (Manhattan)

Burkhard (PR-18B)	1,500
Burkhard (C-1763)	2,330
	3,830

2. University of Kansas (Kansas City & Lawrence)

Bly (SG-1)	Scholar
Bly (C-1916)	9,774
Edelhoch (C-1974)	7,106
Frenkel (CH-8)	6,243
Stahl (C-1987)	13,176
Stowell (INSTR-60B)	25,000
Stowell (DRIR-36B)	10,000
Werder & Hardin (C-1827)	9,869
	81,168

3. St. Margaret's Hospital (Kansas City)

Laing (DRIR-81B)	4,000
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KENTUCKY

1. University of Louisville (Louisville)

Berg (DRIR-160B)	6,700
Hall (MOR-12D)	Ext. time
Kerman & Roseman (DRIR-240)	16,000
Rogers (DRIR-249)	7,000
Rogers (C-1590 C)	6,000
Wiley (DRIR-213A)	5,800
	41,500

2. Ursuline College (Louisville)

Siebert (DRIR-88B)	2,000
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LOUISIANA

1. Alton Ochsner Medical Foundation (New Orleans)

Horvitt & Segaloff (EDC-4)	8,478
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2. Louisiana State University (Baton Rouge)

Kehr (EG-37A)	4,000
Kehr (C-1674 C)	2,700
	7,700

Agency	Research Projects
ACS	Cellular physiology
ACS	Tissue cultures and
NCI	Protein & nucleic acid
ACS	Embryonic differentiation &
	Delayed fertilization

ACS	Protein-dye complexes
NCI	Sulfur containing azo dyes

ACS	Radioisotopes, liver tissue &
NCI	hepatomas
NCI	Macromolecular interactions
ACS	'Ascites tumors'
NCI	Vacuum ultraviolet microspectrophotometry
ACS	Blood, urine & tissue changes
DRMF	
NCI	Immunity and transplants

DRMF	Clinical research
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DRMF	Radioisotopes
ACS	Embryology, nervous system
DRMF	Radioisotope procedures, brain
DRMF	Tumor growth rate-susceptibility
NCI	
DRMF	2-Pyrones & unsaturated lactones

DRMF	Enzyme proteins
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ACS	Biosynthesis of steroids
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ACS	Genetic tumors, plants
NCI	

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3. Tulane University (New Orleans)

	Amount	Agency	Research Projects
Baillif (C-1563 C)	6,000	NCI	Radioactive colloids, reticuloendothelium
Carrera (F-140A)	Fellow	ACS	Nucleases & nucleic acid metabolism
Collins (C-1818)	2,630	NCI	Venereal granulomatous lesions
Farber (SG-2)	Scholar	ACS	Chemical pathology
Krementz (C-1985)	2,166	NCI	Needle biopsy
Kurnick (BGH-7A)	6,500	ACS	Desoxyribonuclease-deso. inhibitor-anti in.sys.
Randolph (PH-18)	3,240	ACS	Ultracentrifuge rotor
Segaloff (INSTR-39E)	60,000	ACS	Hormones
	80,536		

MAINE

Roscoe B. Jackson Memorial Laboratory (Bar Harbor)

	Amount	Agency	Research Projects
Borges (EG-35A)	8,420	ACS	Gene action
Dickie (V-17B)	3,056	ACS	Tissues & mammary gland
Dickie & Woolley (EG-34B)	7,500	ACS	Inherited & physio. factors, susceptibility
Pekete (EG-32B)	5,000	ACS	Uterine environment
Griffen (C-1912)	10,908	NCI	Cytogenetics, cells
Hummel (V-18B)	6,292	ACS	Mammary-tumor-milk agent
Kaliss (CP-40C)	Ext. time	ACS	Lyophilized tissues, homiotransplants,
Kaliss (MOR-24)	7,500	ACS	Homografts, tissue antigens &
Kaliss (C-1594 C)	7,500	NCI	Anti-serums
Kaliss (C-1594 C2)	15,228	NCI	
Little (INSTR-70A)	10,000	ACS	Ovaries, spleen transplants,
Little (H-3C)	6,480	ACS	Mutation, mammary inciter
Little	5,000	EUPF	
Little et al. (#35)	6,500	JCG	
Murray (EG-33B)	5,000	ACS	Inbred stocks
Runner (H-11B)	7,290	ACS	Inherited hormonal activity
Runner (C-362 C4)	30,890	NCI	Mammary gland
Russell (EG-12G)	4,500	ACS	Lethal etc. genes, growth, ovaries
Sawin (EG-1G)	5,047	ACS	Growth, known genetic consti., rabbits
Sawin (C-281 C5)	14,283	NCI	
Snell (EG-14G)	5,940	ACS	Somatic & germinal mutations
Snell & Day (CP-7F)	7,000	ACS	Antigens, resistance, transplants
Speirs & Fuller (C-1895)	12,960	NCI	Eosinophil cell, adrenal & pituitary
White (CP-47B)	3,000	ACS	Plants
White (CP-47Bs)	2,400	ACS	
White (CP-59)	9,072	ACS	
Woolley (H-12B)	4,000	ACS	Endocrine balance
	210,766		

MARYLAND

1. Johns Hopkins University (Baltimore)

	Amount	Agency	Research Projects
Baetjer (C-603 C5)	14,999	NCI	Hexavalent chromate dust
Borysko (DRF-99A)	Fellow	DRMF	Cells, electron microscope
Colowick & Kaplan (MET-5C)	11,000	ACS	Phosphorus compound, respiration
Friedenwald (MOR-22)	6,500	ACS	Corneal epithelium
Gey (PH-13B)	Ext. time	ACS	Adv. physical methods
Grayhack (DRF-104A)	Fellow	DRMF	Prostatic growth
Hellerman (C-392 C6)	16,500	NCI	Chemical processes & metabolism
Kern (F-173)	Fellow	ACS	Propionibacterium
Levine (F-148A)	Fellow	ACS	Biochemical & genetic - paramecium aurelia
Lo (DRF-100A)	Fellow	DRMF	Virus
McElroy (C-377 C5)	4,212	NCI	Drug action, mutations

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<u>Amount</u>		<u>Agency</u>	<u>Research Projects</u>
<u>Johns Hopkins University (Cont'd)</u>			
Morgan (C-393 C6)	14,678	NCI	X-ray fluoroscopic screens
Richter (CS-8D)	Ext. time	ACS	Electrical skin resistance method
Rosenfeld (C-6G)	Ext. time	ACS	Growth promoting principle,
Rosenfeld (C-1841)	4,000	NCI	Embryonic tissue
Rupert (F-137A)	Fellow	ACS	Infrared microspectroscopy, living cells
Scott (EDC-1)	7,500	ACS	Prostatic growth & pituitary gland
Scott (INSTR-45D)	20,000	ACS	
TeLinde & Scott (GPF-3E)	Ext. time	ACS	Experimental endometriosis
Weber (F-183)	Fellow	ACS	Genus clostridium, enzymatic mechanisms
Wilkins (CIE-12E)	13,839	ACS	Hormones, adrenal, gonads & growth
	113,228		
<u>2. University of Maryland (Baltimore)</u>			
Figge (C-845 C4)	9,216	NCI	Melanomas, Mexican axolotls & hybrids
Herbst (C-1965)	3,000	NCI	Putrescine, propanediamine, spermine, etc.-liver
McGafferty (C-1759)	324	NCI	Fetal mouse growth & beta radiation
	12,540		
<u>3. National Cancer Institute (Bethesda)</u>			
Korn (DRF-105A)	Fellow	DRMF	Protein synthesis
<u>MASSACHUSETTS</u>			
<u>1. Amherst College (Amherst)</u>			
Kidder (N-2F)	7,000	ACS	Nutrition, growth & proliferation & survival
<u>2. Boston Dispensary (Boston)</u>			
Steckerl & Schmidt (G-1589 C)	6,150	NCI	Cell ribonucleases, ascites (Ehrlich)
Thannhauser & Schmidt (BGH-18)	6,500	ACS	Phosphoric acid diesters metabolism
	12,650		Nucleic acids binding
<u>3. Boston University (Boston)</u>			
Gensler (CBC-6D)	Ext. time	ACS	Podophyllotoxin & picropodophyllin
Gensler (CH-10)	6,553	ACS	& related compounds
Keefer (INSTR-67A)	18,300	ACS	Tissues & growth activity
Lemon (H-7B)	11,341	ACS	Metabolism, testosterone, human tissues
Lemon (C-1643 C)	5,294	NCI	Anal.meth. 11-ketosteroid content, urine
Lemon & Walker (C-930 C4)	14,500	NCI	Analysis, cellular proteins, origin
Lutz (C-1287 C2)	6,500	NCI	Vascularization neoplasms, cheek
Lutz (C-1644 C)	12,000	NCI	pouch, hamster
Taymor (C-1619 C)	4,000	NCI	Irradiation, P32, DNA, human cervix
	78,488		
<u>4. Children's Cancer Research Foundation (Boston)</u>			
Foley & Farber (C-1921)	8,985	NCI	Dihydrotriazines
Landing (C-1975)	5,400	NCI	Histochemical-endocrine, metabolic & neoplastic diseases of children
	14,385		
<u>5. Children's Medical Center (Boston)</u>			
Farber (C-937 C4)	40,000	NCI	Chemotherapy
Ingraham (C-1608 C)	6,000	NCI	Central nervous system & gamma radiation
Miller (C-1691 C)	13,500	NCI	Mechanism, carcinolytic agents
	59,500		

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	Amount	Agency	Research Subjects
6. <u>Harvard University</u> (Boston & Cambridge)			
Albright (EDC-2)	9,072	ACS	Metabolic interrelations
Albright (C-1887)	15,056	NCI	
Aub (C-4G)	13,000	ACS	Chemical variants &
Aub (INSTR-44E)	100,000	ACS	Cell mitosis
Aub & Nathanson (C-1393 C2)	25,500	NCI	Pituitary, steroid hormones & growth
Cohn (INSP-59B) (deceased)	7,000	ACS	Partial Support U.Lab.Phys.Chem.,Med.& Pub.Heal.
Eaton (C-1657 C)	16,464	NCI	Tissue proliferation & virus growth
Folch-Pi (BGH-11)	7,000	ACS	Brain strandin
Frazier (DRIR-182B)	10,000	DRMF	Paraohenylenediamine & living cells
Griesamer (F-104B)	Fellow	ACS	Respiratory enzymes of epidermis
Hertig (C-1611 C)	2,716	NCI	Cervix, pathogenesis, morphology
Hoagland (SG-12)	Scholar	ACS	Protein synthesis in neoplastic tissues
Ladman (F-147A)	Fellow	ACS	Anterior pituitary gland-histochemical
Munson (EDC-7)	8,814	ACS	Pharmacology of parathyroid
Seligman (C-312 C7)	30,000	NCI	Chemotherapy
Schilling (DRF-94A)	Fellow	DRMF	Metabolism & endocrine activity
Stare & Geyer (C-722 C5)	15,000	NCI	Fat emulsion
Straus (F-182)	Fellow	ACS	Pigment formation & growth of maize endosperm
Thimann (BO-6G)	6,079	ACS	Plant growth
Wetmore & Thimann (BO-16E)	4,500	ACS	Conducting system in vascular plants
Wright (BAF-22)	Fellow	ACS	Lymphoid tissue
Zamecheck & Vitale (C-1323 C3)	15,000	NCI	Gastrointestinal tract pathology
	285,201		Nutrition
7. <u>Marine Biological Laboratory</u> (Woods Hole)			
Armstrong (R-7E)	1,600	ACS	Radiobiology
Armstrong (R-7Es)	5,000	ACS	
	6,600		
8. <u>Massachusetts Eye and Ear Infirmary</u> (Boston)			
Balazs (C-1685 C)	8,500	NCI	Acid mucopolysaccharides & tissue growth
9. <u>Massachusetts General Hospital</u> (Boston)			
Aub (C-558 C5)	17,712	NCI	Chemotherapeutic & phys.agents & cytochemistry
Aub et al. (H-15A)	10,000	ACS	Urinary steroids
Castleman (G-1973)	7,341	NCI	Testes, spermatogenesis
Churchill (INSTR-16F)	120,000	ACS	Basic science & clinical investigations
Churchill (DRIR-187A)	53,700	DRMF	
Colby (J-1649 C)	12,080	NCI	Bladder, cervix
Jones (F-189)	Fellow	ACS	Adenosine triphosphate-coenzyme A-acetate reaction
Lipmann (C-823 C4)	21,583	NCI	Biosynthetic mechanisms
Moldawer (F-150A)	Fellow	ACS	Metabolism & growth
Morgan (C-22)	4,558	ACS	Golgi substance, pancreas homogenates
Nathanson & Engel (#106)	16,760	JCC	Urinary steroid metabolites
Scott (BGH-17)	6,500	ACS	Spectroscopic investigation, molecular inter-
Scott (SG-5)	Scholar	ACS	action, growth
Sweet	23,100	AEC	Isotopes, brain, neurosurgical relief of pain,
Sweet (DRIR-161A)	8,750	DRMF	Dev. stereotactic methods
Zamecnik & Aub (E-16E)	6,000	ACS	Protein metabolism
	308,084		
10. <u>Massachusetts Institute of Technology</u> (Cambridge)			
Bear (C-18B)	7,560	ACS	X-ray diffraction connective tissue
Buchanan (DRIR-239)	11,000	DRMF	Enzyme systems
Fitzgerald (F-102B)	Fellow	ACS	Protein fiber formation
Remy (F-136A)	Fellow	ACS	Biosynthesis of nucleotides
Rothstein (DRF-145)	Fellow	DRMF	Ossilated nerve proteins
Spiro (DRF-123A)	Fellow	DRMF	Ultrastructure of mitochondria
	18,560		

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	Amount	Agency	Research Subjects
11. <u>University of Massachusetts</u> (Amherst) Woodside (C-1661 C)	8,375	NCI	Chemotherapy
12. <u>New England Center Hospital</u> (Boston) Alisangco (DRF-147) Dameshek (CPB-16C) Desai (DRF-135) Kumminos (DRF-97A) Patterson (F-180) Pratt (CS-16A)	Fellow 6,696 Fellow 6,674 Fellow 4,752 11,448	DRMF ACS DRMF DRMF ACS ACS	Serum proteins of patients, leukemia, etc. Hemolytic antibodies & leukemia, etc., hemolytic anemia White cell antibodies & leukemia Immunohemolytic anemia & leukosarcoma Cell growth & host resistance Pancreas, urinary enzymes
13. <u>New England Deaconess Hospital</u> (Boston) Gates (C-1844) Hicks (C-1042 C3) Sommers (C-1936) Warren (DASP-74)	8,573 9,774 6,674 10,000 35,021	NCI NCI NCI ACS	Intercellular substances tumor stroma Tissue metabolism nervous system Human cancer growth, hamster Expenses, Polaroid Color Transplanting Ultraviolet Microscope-Dr. Ruth Graham, etc.
14. <u>Peter Bent Brigham Hospital</u> (Boston) Goetz (DRF-73A) Haydar (DRF-144) Sturgis (H-17A)	Fellow Fellow 4,816 4,816	DRMF DRMF ACS	Adrenalectomy & prostate, hyperinsulinism Adrenal steroid metabolism Steroid hormones & endometrial nucleic acids
15. <u>Scientific Specialties Corporation</u> (Boston) "Microscopes" (INSP-73) "Microscopes" (DRIR-252)	75,000 10,000	ACS DRMF	Purchase, color translating ultraviolet microscope
16. <u>Thorndike Memorial Laboratory</u> (Boston) Freinkel (F-168)	85,000 Fellow	ACS	Tissue iodide accumulation
17. <u>Tufts College</u> (Medford & Boston) Bernfeld (C-1680 C) Bonner (C-1958) Christensen (C-1268 C2) Davidson (F-191) Fishman (C-915 C4) Fishman (C-915 C5) Fishman (C-1964) Fishman & Bernfeld (E-39B) Homburger (INSTR-31F) Homburger (DRIR-43C) Kasdon (C-1978) Shen (CPB-15D)	6,000 4,762 16,330 Fellow 10,000 10,000 5,000 4,800 50,000 9,401 6,856 Ext. time 123,149	NCI NCI NCI ACS NCI NCI NCI ACS ACS DRMF NCI ACS	Plasma proteins, micro-electrophoresis Glucuronic acid metabolism, humans Amino acid assimilation & cells Phosphoproteins, prostatic acid phosphatase B-glucuronidase & growth Biochem. stud. prostatic acid Phosphatase Purified B-glucuronidase Enzymatic synthesis glucuronidase Plasma proteins, mice Vaginal beta-glucuronidase Anemia
18. <u>Vincent Memorial Hospital</u> (Boston) Graham (C-1810)	11,053	NCI	Radiation & sensitization response-SR
19. <u>Wellesley College</u> (Wellesley) Jones (C-821 C5)	5,956	NCI	Mammary glands, mice Tests on paramecia
20. <u>Worcester Fdn. for Experimental Biology</u> (Shrewsbury) Pincus (EDC-8) Pincus (INSTR-63B) Pincus (DRIR-61C)	10,933 18,600 10,700 40,233	ACS ACS DRMF	Biogenesis androgens & estrogens Steroid metabolism

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MICHIGAN

	Amount	Agency	Research Subjects
1. <u>Detroit Institute of Cancer Research</u> (Detroit)			
Albert & Johnson (BCH-1A)	5,000	ACS	Chromosomes, phosphorus meta. & endocrine organs
Guthrie (C-1872 R)	7,859	NCI	Ovarian cells
Johnson & Albert (C-928 C4)	8,125	NCI	Cells, phosphoprotein, mammalian tissues
Johnson & Albert (C-1692 R)	4,300	NCI	
Raut	35,739	EUPF	Chemotherapy
Simpson & Scott (INSTR-17F)	50,000	ACS	Integrated stud., clin. & exper.
	111,023		
2. <u>Michigan State College</u> (East Lansing)			
Tukey & Lucas (CH-17)	8,000	ACS	Assay, substances, cell behavior
3. <u>University of Michigan</u> (Ann Arbor)			
Bauer (C-1719 R)	7,425	NCI	Tumor glutathione
Bethell (C-1994)	11,381	NCI	Labile methyl, leukemic, non-1., & pteroylglutamic acid
Furstenberg (INSTR-69A)	15,000	ACS	Nature, detection, cure
Hodges	3,000	EUPF	Clinical records
Miller et al. (C-1896)	5,000	NCI	Estrogen
Susman (MET-11B)	2,160	ACS	Ascospores of neurospora
Sutherland (C-1559 C2)	7,000	NCI	Infrared spectroscopy & protein molecules
Vial (C-1835)	14,110	NCI	Antibody-isotope complex, human
	65,076		
4. <u>Siena Heights College</u> (Adrian)			
Stinson (PH-12B)	1,188	ACS	Ultraviolet absorption & nucleic acid deriv.
5. <u>Wayne University</u> (Detroit)			
Djerassi (EDC-3)	6,782	ACS	Isomers, analogs steroid hormones

MINNESOTA

1. <u>Concordia College</u> (Moorehead)			
Werth (C-1606 C)	216	NCI	Benzo(c)phenanthrene & derivatives
2. <u>University of Minnesota</u> (Minneapolis)			
Barnum (V-5G)	6,000	ACS	Mammary glands, mice
Bittner (EG-17G)	5,000	ACS	Mammary cancer & genetics
Bittner (EG-18G)	10,000	ACS	Tumor milk agent
Bittner	20,000	EUPF	
Cohen (C-1330 C2)	10,495	NCI	Steroidal conjugation mechanisms
Diehl (INSTR-49D)	75,000	ACS	Insti. research, U. Minn.
Gutman (MET-21)	4,000	ACS	Carcinogenic amines & metabolism
Hitchcock (C-298 C7)	15,000	NCI	Gastric cancer, methylcholanthrene
Hitchcock (C-1218 C2)	3,980	NCI	induction, stomach
Kolthoff (C-721 C4)	15,660	NCI	Metal ions & sulfhydryl compounds
Kolthoff (C-721 C5)	15,000	NCI	Blood sera
Reed (EG-24E)	6,925	ACS	Genetics, breast, human
Syvertson (V-6G)	4,800	ACS	Host cell & virus, immunization
Syvertson (C-725 C4)	15,000	NCI	leukemia & mammary - mice
Wangenstein (DRIR-99B)	15,000	DRMF	"Second-Look" operations
Wild (C-1244 C2)	21,616	NCI	Ultrasonic pulses & tissue density changes
Zimmerman (SG-17)	Scholar	ACS	Endocrine physiology & metabolic balance
	243,476		

MISSOURI

1. <u>Midwest Research Institute</u> (Kansas City)			
Goodson (C-802 C4)	31,270	NCI	Animal assay for treatment
Goodson (C-1816)	3,051	NCI	

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	Amount	Agency	Research Subjects
2. <u>University of Missouri</u> (Columbia)			
Faberge (C-990 C4)	1,725	NCI	Genetic mutations & ultraviolet radiation
Novitski (C-1578 C2)	9,167	NCI	Chromosomes
Shaver (C-1989)	5,147	NCI	Cleavage in frog egg
	16,039		
3. <u>St. Louis University</u> (St. Louis)			
Bauer (DRIR-189A)	9,000	DRMF	Rapid freezing, virus & non-virus
Luyet (CP-53A)	5,075	ACS	Cell survival & frozen tissue
Luyet (#103)	3,500	JCC	Red cells, frozen blood & ice formation
Seibert (DRF-121)	Fellow	DRMF	Steroid metabolism & enzymatic activity
	17,575		
4. <u>Washington University</u> (St. Louis)			
Allen	9,000	AEC	Gamma ray therapeutic agent
Berg (F-158A)	Fellow	ACS	Enzymes & phosphate di-ester bond
Commoner (V-9D)	6,561	ACS	*Reduplication tobacco mosaic virus, growth processes
Graham (DRIR-251)	12,700	DRMF	*Tobacco smoking & lung cancer
Gutsche (CH-11)	6,966	ACS	Colchicine & related compounds
Hamburger & Levi-Montalcini (C-1801)	13,100	NCI	Sarcomas(mice)& nervous system chick embryo
Lowry (C-11E)	13,895	ACS	Cytochemistry nervous system
Cori (INSTR-32F)	60,000	ACS	Growth
Roberts (C-1245 C2)	13,000	NCI	Free amino acids, peptides & nitrogen meta. tissues
Silberberg & Silberberg (EDC-9)	4,000	ACS	Hormones, mice
Velick (E-25D)	7,100	ACS	Enzymes
Weiss (DRF-92A)	Fellow	DRMF	Orcein, elastin reaction
	146,322		

MONTANA

<u>Montana State University</u> (Missoula)			
Loran (DRIR-192A)	5,300	DRMF	Oncolytic effect NDBS fraction podophyllin on tumor tissue

NEW HAMPSHIRE

<u>Dartmouth College</u> (Hanover)			
Tyson (DRIR-253)	7,000	DRMF	Pulmonary function radiation fibrosis

NEW JERSEY

1. <u>Princeton University</u> (Princeton)			
Bonner (MOR-16C)	1,000	ACS	Cell differentiation amoeboid slime molds
Fankhauser (MOR-15C)	4,500	ACS	Dev., grow., & fertility polyploid salamanders
Jacobs (BQ-18D)	2,160	ACS	Cell differentiation vascular plants
	7,660		
2. <u>Rutgers University</u> (New Brunswick)			
Allison (INSTR-51D)	17,812	ACS	Tissue equilibrium & metabolic interrelations
Allison & Leatham (CP-23E)	8,856	ACS	Experimental production tumors
Crossley (CH-5A)	6,000	ACS	Chemotherapy
Leatham (EDC-6)	6,156	ACS	Abnormal ovarian growth
	38,824		

NEW MEXICO

1. <u>University of New Mexico</u> (Albuquerque)			
Daub (C-1595 C)	3,672	NCI	1-methyl, 7-meth., 8-meth. & 10-methyl-3, 4-benzpyrene

2. Highlands University (Las Vegas)
Robins (DRIR-260)

Amount
4,800

Agency

Research Subjects

DRMF Imidazo(c)pyridines, potential purine antagonists

NEW YORK CITY

1. American Museum of Natural History
Breder (DRIR-39D)
Breder (DRIR-39E)

10,000
10,000
20,000

DRMF

Environmental & endocrine control

DRMF

Cell proliferation

2. College of the City of New York
"Society for Experimental Biology & Medicine" (DRIR-143A) 150

DRMF

Pub. cancer articles

3. Columbia University

Bloch (F-184)
Chargaff (BCH-3A)
Curth (C-1603 C)
Davidson (F-146A)
Dische (PR-16C)
Fiala (C-21A)
Frantz et al. (C-1981)
Gellhorn (DRIR-56C)
Gellhorn (C-1386 C2)
Gellhorn & Hirschberg (C-1894)
Gluecksohn-Waelsch (G-2)
Gold (F-159A)
Godman (#114)
Gray (DRIR-243)
Graff
Graff & Haagenensen (C-1794)
Gusberg (C-1793)
Habif (DRIR-244)
Hudson (BCH-6A)
Hudson & Reiner (C-1639 C)
Hyman (DRIR-255)
Jailer (S-12D)
Lattes (DRIR-247)
Levy (ENV-2B)
Lieberman (C-1918)
Murray & Chargaff (DRIR-162B)
Oppenheimer (C-1620 C)
Quimby (R-16A)
Rappleye (INSTR-18F)
Rittenberg (IS-2G)
Rittenberg et al. (CIE-3G)
Ryan (BC-13F)
Shemin (MET-24)
Shils & Shapiro (C-1783)
Stork (CH-16)
Taylor (C-1797)
Taylor & Lieberman (DRIR-215A)
Wallace (DRF-139)

Fellow
7,500
7,117
Fellow
6,000
Ext. time
3,240
79,110
10,000
8,083
3,070
Fellow
2,980
100
16,000
10,000
7,500
15,000
6,000
5,000
7,500
4,000
3,200
Ext. time
16,300
6,480
32,076
8,527
125,000
10,935
15,000
7,000
6,000
5,672
7,000
6,884
23,500
Fellow
471,774

ACS Desoxyribonucleic & nuclear prot., mitotic cycle
ACS Nucleoproteins
NCI Acanthosis nigricans
ACS Chemotherapy & nucleic acid turnover in tumors
ACS Proteins (prosthetic) & growth
ACS Carcinogen accumulation (mitochondria)
NCI Thyroid (Long-Evans rat) internal & ex. radiation
DRMF Purines & pyrimidines
NCI in chemotherapy
NCI Chemotherapy, brain tumors, human
ACS Genetics, embryonic death, early pregnancy
ACS Steroidal patterns, adrenal virilism & cortisone
JGC Mitotic cycle & antimetabolites
DRMF Spon. lecture in U.S., Dr. L.H. Gray, Great Brit.
EUFF Growths in animals
NCI Anti-leukemia protective factor
NCI Radiosensitivity, cervical, humans
DRMF Chemotherapy
ACS Acid Phosphatase, human prostatic & adenoma
NCI Prostate, enzymatic glutamine synthesis
DRMF Iron metabolism & neoplastic disease
ACS Metabolism, estrogens, adrenal steroids & proteins
DRMF Mitotic cycle & antimetabolites - cytochemical
ACS Oral neoplasms, tongue
NCI Urinary 17-ketosteroids & cortisone, hydrocortisone
DRMF Mitosis poisons
NCI Invest. imbedding plastic films
ACS Radiation dosage
ACS Stud., Insti. Cancer Research & Francis Delafield Hosp.
ACS Metabolism, proteins, tissues
ACS Isotope technique, humans
ACS Biochemical mutants microorganisms
ACS Biosynthesis porphyrins
NCI Chemotherapy, vitamin content of host
ACS Colchicine & related tropolone systems
NCI Human ovary, gynecologic neoplasms
DRMF Cyto. & biochemistry, neoplasms, human
DRMF Steroidal excretion patterns
in adrenal virilism

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	Amount	Agency	Research Subjects
3. <u>Cornell University</u>			
Cattell & Kensler (BI-2F)	7,500	ACS	Azo dyes, liver, rats
Engle (C-1905)	8,310	NCI	Multiple myeloma, abnor. proteins & plasma cells
Ferguson (C-1917)	2,862	NCI	Colchicine, biological & pharmacological
Ferguson (C-1992)	9,134	NCI	
Kidd (CPB-18B)	Ext. time	ACS	Structure changes & antibodies, cytotoxic agents
Kidd (V-2G)	15,000	ACS	Virus papillomas, cytological & biological
Papanicolaou (INSP-58B)	48,000	ACS	Exfoliative secretions breast
Riker (MA-7E)	Ext. time	ACS	Pharmacology compounds & mitosis & growth
	90,806		
4. <u>Francis Delafield Hospital</u>			
"Genetics Society of America" ¹ (DRIR-242)	1,000	DRMF	Contributions send members Int. Cong. Genetics Italy
5. <u>Fordham University</u>			
Berger (C-492 C5)	2,160	NCI	Polyploidy & diploid plants
Brown (C-1390 C2)	5,616	NCI	Heterocyclic analogs compounds
Brown (C-1821)	4,050	NCI	Sex hormones
Cerecedo (DRIR-41B)	7,200	DRMF	Nucleic acids & growth, purine & amino acid
Cerecedo (C-1370 G2)	6,700	NCI	composition tissues
	25,726		
6. <u>Harlem Hospital</u>			
Wright (DRIR-50C)	15,000	DRMF	Tissue culture
Young (C-1607 C)	9,913	NCI	Triethylene melamine, antibiotics, folic acid
	24,913		antagonists, achromycin, ACTH
7. <u>Haskins Laboratories</u>			
Rutner (CP-57)	4,700	ACS	Nutrition, lipid growth, microorganisms
Zahl & Albaum (C-1622 C)	9,115	NCI	Blood & tissue levels nucleotides
	13,815		
8. <u>Memorial Center for Cancer & Allied Diseases</u>			
Bieseke (C-678 C5)	11,100	NCI	Tissue culture
Bodansky (C-1443 C2)	12,085	NCI	Adrenal & other steroid & biochem. blood, tissues
Bodansky & Randall (C-1694 C)	15,000	NCI	Composition intracellular phase, surgery, human
Brown (C-471 C5)	28,336	NCI	Nucleo-proteins & growth
Burchenal (C-679 C5)	17,500	NCI	Chemotherapy exper. leukemia
Changus (C-1971)	4,860	NCI	Histochemical differentiation bone tumors
Fath (DRF-46A)	Fellow	DRMF	Thyroid-adrenal relationships
Fox (DRF-124A)	Fellow	DRMF	Nucleic acid biosynthesis, nucleotides & ribofuranosyl nuc.
Gallagher (S-4F)	30,600	ACS	Steroid excretion
Gallagher (C-440 C4)	39,009	NCI	Biochemical & chemical inves. steroids
Guthrie (C-1848)	9,681	NCI	Chem. genetics bacteria & nucleic acid metabolism
Hansbury (F-169)	Fellow	ACS	Thyroid stimulating hormone (TSH) & lipid metabolism
Hamilton (C-1813)	5,899	NCI	Nucleic acids & compounds & human leukocytes
Kappas (F-107B)	Fellow	ACS	Steroid metabolism
Karnofsky (C-675 C5)	12,733	NCI	Neoplastic tissue, chick embryo, Hodgkin's disease
Koch (SG-4)	Scholar	ACS	Hemoglobin metabolism & ionizing irradiation
Laughlin & Nickson (R-21)	8,100	ACS	Cell differential sensitivity & radiation
Li (DRF-140)	Fellow	DRMF	Hypophysectomy & hypothalamic irradiation, mammary gland
Mellors (DRIR-258)	9,500	DRMF	Quan. analy. cell interference microscopy
Murphy (DRF-117A)	Fellow	DRMF	Biochem., resistance A-methopterin
Nickson & Escher (DRIR-72B)	10,000	DRMF	Steroid changes, hormone therapy, radiation, mammary gland
Nickson & Laughlin (DRIR-226A)	22,000	DRMF	High energy radiation, humans
Nickson & Seal (C-1579 C)	13,000	NCI	Prog. eval. rotational therapy to 250 KV
Ortega (DRF-141)	Fellow	DRMF	Microscopy, humans
Pearson (DRIR-259)	10,000	DRMF	Altered hormonal environ. & breast cancer
Phillips (C-415 C5)	38,921	NCI	Program chemotherapeutic agents
Phillips	20,000	EUFP	
	950,160		

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	Amount	Agency	Research Subjects
<u>Memorial Center (Cont'd)</u>			
Poppell (DRF-101A)	Fellow	DRMF	Cardiopulmonary function & radical surgery
Randall & Fortner (C-1877)	5,522	NCI	Etiology, gallstones
Rawson et al. (EEP-14G)	11,000	ACS	Thyroid, biology
Rawson & Pearson (C-925 C5)	29,772	NCI	Tissue catabolism & metabolism, humans
Rhoads (INSTR-10G)	350,000	ACS	Steroid cancer chemotherapy
Rhoads (DRIR-103B)	7,500	DRMF	Damon Runyon Research beds, children
Rhoads (DRIR-150A)	40,000	DRMF	Damon Runyon Research Ward
Rhoads (DRIR-219A)	34,000	DRMF	Clin.Stud., viruses
Rhoads & Day (DRIR-223A)	24,300	DRMF	Respiratory, upper gastro-intestinal & environment
Rhoads et al. (DRIR-208A)	13,000	DRMF	Pathologic physiology, prostatic cancer
Rhoads & Marshall (DRIR-76A)	15,000	DRMF	Hormone therapy, vesical carcinoma, dogs
Rhoads & Rawson (DRIR-218A)	12,000	DRMF	Serum iodine, serum protein & thyroid & radioiodine
Rhoads & Richardson (DRIR-241)	7,200	DRMF	Histopathology, hypothysectomized animals fed carcino.
Rhoads et al. (C-1889)	40,000	NCI	Clin. eval. chemotherapeutic agents
Richardson et al. (C-1809)	10,000	NCI	Inhibitory actions chemical carcinogens
Roberts (DRF-118A)	Fellow	DRMF	Metabolic balance, surgical patients
Sonenberg (SG-9)	Scholar	ACS	Pituitary physiology
Toolan (#108)	15,000	JCC	Human tumors in animals
West (SG-16)	Scholar	ACS	Endocrine factors
Woolley (C-1796)	17,542	NCI	Steroids & cancer control
9. <u>Montefiore Hospital</u>	950,160		
Laszlo (DRIR-246)	10,000	DRMF	Metabolic studies, patients
Laszlo (C-1540 C2)	23,170	NCI	
	33,170		
10. <u>Mount Sinai Hospital</u>			
Hollander (C-228 C7)	12,997	NCI	Gastric mucus secretion, gastric can. & peptic ulcer
Kremen (C-1876)	8,154	NCI	Nutritional adjustment & extensive intestinal resections
Sobotka (C-1791)	8,915	NCI	Lipoid complexes serum albumin
	30,066		
11. <u>New York Medical College</u>			
Neuberg (C-16G)	4,000	ACS	Nucleic acid complexes & growth
12. <u>New York University</u>			
Brendler (H-5B)	5,000	ACS	Nutrition, endocrine therapy, prostatic
Brendler (C-1599 C)	5,994	NCI	
de Bodo (EEP-15D)	11,000	ACS	Adrenal cortical hormones & pituitary
Goldsmith (N-14B)	3,780	ACS	Nucleic acid metabolism, drosophila melanogaster
Gordon (CPB-19B)	5,870	ACS	Endocrine factors, blood formation & destruction
Harnly (DRIR-256)	9,500	DRMF	Larval fluids of drosophila melanogaster
Harnly & Kopac (C-1580 C)	3,600	NCI	Nutrition, temperature, tumor genes, drosophila
Hirshfield (CP-52A)	4,500	ACS	Nucleus cytoplasm & n.c. complex & cell growth & differen.
Landauer (DRF-131)	Fellow	DRMF	Pressure-temperature, energetics Sol-gel react., cell div.
Levy (BCH-15)	5,500	ACS	Chemical structure proteins
Marsland (C-807 C4)	2,451	NCI	Mechanisms cell division,
Marsland (C-807 C5)	2,986	NCI	Pressure-temperature study
Mateyko (DRF-125A)	Fellow	DRMF	Intracellular stratification, histochem. & cytochem. cells
Mulholland (INSTR-61B)	30,000	ACS	Supporting & integrating projects at Center
Nelson (DRIR-138A)	81,680	DRMF	*Respiratory, environment, inhaled materials
Ochoa (E-10F)	7,560	ACS	Enzyme systems, biol. oxidations & synth., animal tissues
Ratner (E-21E)	6,750	ACS	Enzyme mech., amino nitrogen, urea formation
Sheehan (DRIR-247)	5,679	DRMF	Equipping operating room
Slautterback (F-153A)	Fellow	ACS	Proteolytic activ. microsome fract., neoplas. tissue
Sulzberger (C-1379 C2)	10,000	NCI	Immunologic & allergic changes
Taylor (CP-41B)	4,500	ACS	Tissue survival after freezing
	206,350		

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13. New York Zoological Society
Gordon (C-297 C5)

14. Presbyterian Hospital
Golden et al. (C-1756)
Klegerman (DRIR-235)

15. Rockefeller Institute
Marmur (F-178)

NEW YORK STATE

1. Albany Medical College (Albany)
Wright & Wolfe (C-1105 C3)

2. Basset Hospital (Cooperstown)
Hanks (C-1027 C4)

3. University of Buffalo (Buffalo)
Bloom (C-1853)
Carruthers (BCH-2A)
Kimball (INSTR-76)
Lowe (C-1693 C)
Whitbasky (DRIR-137A)

4. Carnegie Institution of Washington (Cold Spring Harbor)
Demerec (EG-21F)

5. Cornell University (Ithaca)
Singer (MCR-19A)

6. Maimonides Hospital (Brooklyn)
Friedgood (C-1383 C2)

7. Nassau Hospital (Mineola)
Ponder (CPB-14D)

8. New York State College of Agriculture (Ithaca)
Smith & Srb (C-1256 C3)

9. Polytechnic Institute of Brooklyn (Brooklyn)
Barker (DRIR-200A)

10. Research Fdn. of the State Univ. of New York (Brooklyn & Syracuse)
Burlington (C-1875)
Schulman (C-1852)
Tepperman & Tepperman (EDC-11)
Weiss (C-1800)
Westerfeld (N-10D)

Amount	Agency	Research Subjects
17,200	NCI	Pigment cell growth
135,975	NCI	Multimillion volt x-ray & electron beam therapy
2,954	DRMF	Graphics & localizing radium implants
138,929		
Fellow	ACS	Bacterial transformation
11,707	NCI	Etiology spontaneous mammary tumors Milk agent, rats
4,470	NCI	Course: Principles, techniques, & applications of tissue culture
13,825	NCI	Porphyryn, hemoglobin, iron & copper metabolism
5,000	ACS	Polarographically reducible substances
15,000	ACS	Institutional research, U. Buffalo
8,000	NCI	Cortisone, nucleic acid metabolism, liver
12,960	DRMF	Properties, tissues
54,785		
7,400	ACS	Mutagens
4,000	ACS	Nerve & regeneration of amputated extremity, regeneration process, salamander, etc.
4,000	NCI	Nitrofurans
5,000	ACS	Hemolytic material & tissue
6,000	NCI	Chromosome structure, gene mutation & certain chemicals
15,000	DRMF	Protein structure
1,000	NCI	Hormones, peptide bond synthesis
5,848	NCI	Biosynthesis of porphyrins
4,752	ACS	Cell adaptation, mammalian tissue
4,590	NCI	Amino groups, blood & hematopoietic tissues
6,000	ACS	Diet, xanthine oxidase etc.
22,190		

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	Amount	Agency	Research Projects
11. <u>University of Rochester</u> (Rochester)			
Dounce (C-994 C3)	5,500	NCI	Biochem., cell nuclei, enzyme systems
Holtfreter (CP-8F)	Ext. time	ACS	Differentiating cells
Keutmann & Burton (C-1003 C3)	13,000	NCI	Paper partition chromatography to steroid hormones, human
Keutmann & Waterhouse (C-739 C5)	25,493	NCI	Metabolism
Miller (#101s)	22,500	JCC	Protein synthesis & neoplastic growth
Morton & Keutmann (INSTR-57G)	23,185	ACS	Tumor host relationships
Stolz (#111)	10,000	JCC	Oxidation capacity, hormone-stimulated uterus
Tobin (C-1946)	3,684	NCI	Lymphatics, human lungs
	103,362		
12. <u>St. Johns University</u> (Brooklyn)			
Lilly (CP-58)	3,000	ACS	Nutrition & growth, protozoa
13. <u>Society for Study of Development and Growth</u> (Brooklyn)			
Boell (C-1970)	1,000	NCI	12 Sympos., Soc., Study of Development & Growth
Nickell (Treasurer) (INST-3E)	1,000	ACS	Annual " " " " " " " "
	2,000		
14. <u>Trudeau Foundation</u> (Saranac Lake)			
Vorwald (ENV-4A)	13,618	ACS	Adenocarcinoma, lung, rats, & beryllium
Vorwald & Pratt (DRIR-75B)	9,265	DRMF	Industrial dust & lung cancer
	22,883		
15. <u>Vassar College</u> (Poughkeepsie)			
Kuntz (R-20)	9,000	ACS	Irradiation & neoplasia on nucleolytic enzymes of lymphoid tissues, mice
16. <u>Waldemar Medical Research Foundation</u> (Brooklyn)			
Molomut (CP-54A)	4,892	ACS	Prior allergic conditioning
Spain & Molomut (C-1658 C)	9,000	NCI	Alteration of response cancer grafts, inter-abdominal sarcoma & heterologous tumor grafts
	13,892		
<u>NORTH CAROLINA</u>			
1. <u>Duke University</u> (Durham)			
Anlyan (C-1439 C2)	3,996	NCI	Tissue antigen, gastric secretions, stomach
Beard (V-14D)	12,500	ACS	Avian leukosis virus
Beard (C-972 C4)	20,000	NCI	
Eckert (SG-11)	Scholar	ACS	Avian leukosis virus
Engel (EEP-10E)	6,000	ACS	Hormones, metabolism, steroid hormones
Hobbs (DRIR-186A)	37,000	DRMF	Smoke aerosols
McKinney (F-125B)	Fellow	ACS	Metabolism, human bone marrow & chemicals, radiant energy
Naylor (CP-42B)	4,498	ACS	Maleic hydrazide - plant growth inhibitor
Rundles (C-1843)	4,752	NCI	Triethylene melamine therapy & serum proteins, humans
Smith (C-1636 C)	2,513	NCI	Testicular tumors
Werk (F-192)	Fellow	ACS	Ketone metabolism, human
Willet (DRF-39)	Fellow	DRMF	Abnormal proteins, multiple myeloma
	91,259		
2. <u>University of North Carolina</u> (Chapel Hill)			
Bunce (C-1867)	2,700	NCI	P-32 uptake by bladder tumors
Cutter (CP-38C)	3,564	ACS	Isolated endosperm nuclei & nucl. cytoplas. relationships
Hooker (#52)	4,000	JCC	Testicular tumors, mice
Irvin (BOH-13)	6,000	ACS	Nucleoproteins & nucleic acids, tissues, interaction molecules
Thomas & Peters (C-1915)	5,477	NCI	Radiogold, lymphatic, human
	21,741		
3. <u>Wake Forest College</u> (Winston Salem)			
Swanson (E-37B)	3,200	ACS	Intermediary metabolism, washed particles liver

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NORTH DAKOTA

N. D. Agricultural Experimental Station (Fargo)
Sleeper (MET-25)

Amount	Agency	Research Subjects
1,800	ACS	Oxidative metabolism of starch & cellulose by actinomycetes

OHIO1. University of Cincinnati (Cincinnati)

Freiman (C-1836)
 Schiff (DRIR-245)
 Vilter (BCH-19)

3,240	NCI	Enzyme activation in tissue
9,700	DRMF	Physiochemical prop., gastric mucus in gastric c.
6,000	ACS	Nuclease activity, cells, human leukemia & inhibitors
18,940		

2. Ohio State University (Columbus)

Doan (INSTR-56C)
 Doan (C-1560 C)
 Hayes & Knouff (C-1817)
 Meyers (C-1899)
 Morton
 Schlumberger (C-1567 C)
 Towbin (C-1623 C)
 von Haam (C-1683 C)

15,000	ACS	Coordinated insti. research program
53,946	NCI	Total body irradiation on sub-human primates
4,536	NCI	Acetal lipids in cells
15,500	NCI	Artificial radioisotopes for therapy
25,000	AEC	Radioisotopes & therapy
3,626	NCI	Study of reaction to injury
5,391	NCI	Transplants, human tumors, cortisone, total body radiation
5,380	NCI	Biological testing carcinogenic hydrocarbons
128,379		

3. Western Reserve University (Cleveland)

Benue (F-163)
 Cantoni (E-40B)
 Dobyns (CS-13B)
 Hirschmann (C-1679 C)
 Kaufman (C-1735)
 Leuchtenberger (C-1814)
 Sayers (CIE-8F)
 Simeone (C-1571 C)
 Weisberger (CPB-17B)
 Weisberger (C-1678 C)
 Wood (IS-5G)

Fellow	ACS	Thyroid, radioiodine
10,000	ACS	Enzymatic mechanisms in transmethylation
8,500	ACS	Thyroid cancer
6,324	NCI	Adrenal steroids, chemistry & metabolism
13,371	NCI	Animal viruses, animal & human tumors
12,074	NCI	Mitosis inducing properties of tumors
12,852	ACS	Pituitary adrenocorticotrophic hormone
4,408	NCI	Gastro-intestinal tract, hydrocarbons
Ext. time	ACS	Cysteine & related compounds
5,934	NCI	in leukopoiesis
12,000	ACS	Isotopic tracer studies biochem. problems re physiology cells
85,463		

OKLAHOMA1. Oklahoma Medical Research Institute (Oklahoma City)

Kochakian (INSTR-62B)
 Kochakian (C-1954)
 Rebell & Lamb (C-1930)
 Reifenstein (C-1564 C)

31,900	ACS	Growth
10,284	NCI	Androgens & tissue enzymes
4,644	NCI	Systemic mycoses in mice with lymphomas
24,732	NCI	Agents altering tissue growth rate of tissues, man and animals
71,560		

2. University of Oklahoma (Oklahoma City)

Everett (C-1633 C)
 Hopps (C-1926)
 Shetlar (CS-17A)

4,500	NCI	Mucopolysaccharides associated proteins, tissue
6,025	NCI	Factors inhibiting neoplastic growth
4,940	ACS	Serum polysaccharide tests
15,465		

OREGONUniversity of Oregon (Portland)

Clancy (CP-61)
 Fitzpatrick et al. (DRIR-257)
 Grossman (C-1206 C2R)
 Lerner (MET-1C)

2,000	ACS	Transplants, nutrition, phenotype, melanotic tumors eye
17,000	DRMF	Metabolism of melanomas
4,060	NCI	Steroid hormones, in vitro incor. C ¹⁴ glycine, liver protein
4,750	ACS	Melanomas, metabolism & chemotherapy
27,810		

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PENNSYLVANIA

	Amount	Agency	Research Subjects
1. <u>Children's Hospital of Philadelphia</u> (Philadelphia) Gostling (BAF-21)	Fellow	ACS	Biological investigation virus hypothesis
2. <u>Hahnemann Medical College & Hospital</u> (Philadelphia) Briody (VI-2) Oesper (MET-15A) van Dyke (C-1878) Wase & Boyd (C-1881)	8,000 4,950 6,600 8,000 27,550	ACS ACS NCI NCI	Viruses, Krebs 2 "ascites tumor", mice Oxidative reaction of glycolysis Thyroid & thymic neoplasia Biochemical relations, 2-acetylaminofluorene & riboflavin during chemocarcinogenesis
3. <u>Immaculata College</u> (Immaculata) Suter' (DRIR-196A)	1,500	DRMF	Spleen extract action on fatty acid metabolism of tissues
4. <u>Institute for Cancer Research</u> (Philadelphia) Briggs (C-913 C3) Greene (CBC-2F) Greene (PR-13D) Diller (CP-45B) Hauschka & Levan (C-1663 C) Lavine (C-1252 C3) Patterson (C-1253 C3) Reimann (INSTR-21F) Schultz (EG-29D) Schultz (C-1613 C) Stekol (C-1251 C3) Stekol (C-1251 C3s) Stekol (C-1251 C4) Toennies (C-1249 C3) Toennies (C-1479 C2) Weinhouse (E-30D) Weinhouse (C-1299 C3) Weinhouse (C-1476 C) Wenner (DRF-110A)	8,352 Ext. time Ext. time 3,000 12,225 2,700 19,336 150,000 9,000 15,778 13,900 2,500 17,580 11,660 14,850 7,500 20,000 10,000 Fellow 318,381	NCI ACS ACS ACS NCI NCI NCI ACS ACS NCI NCI NCI NCI NCI NCI ACS NCI NCI Fellow DRMF	Nucleus & embry. differen. & carcinogenesis Polysaccharides, immunization, Hydrocarbons Fungi Ascites tumors chromosomes & immunogenetic prop. Sulfur compounds Crystallographic tech. & struc. & prop. bio. systems Research in application of various techniques Genes, nutrition, drosophila melanogaster Heterochromatic chromosomes-cytochemical Metabolic interconversion amino acids, animals Sulfur containing nucleo-proteins Folic acid activity blood Intermediary cell metabolism, isotopic tracers Intermediary metabolism, tissues Oxidative metabolism neoplastic tissue
5. <u>Jefferson Medical College</u> (Philadelphia) Miller & Turner (C-1585 C) Paschke & Cantarow (C-1845) Rutman et al. (C-1307 C3) Schepartz (C-1803 C3) Tocantins (C-1931)	8,748 5,616 7,500 4,914 14,000 40,778	NCI NCI NCI NCI NCI	Myelokentric & lymphokentric acids Antimetabolites & chem. induced carcinogens Uracil, nucleic acid formation, acetaminofluorene, rats Phenylalanine & tyrosine in vitro metabolism Hemostasis neoplasms, blood-forming organs
6. <u>Montefiore Hospital</u> (Pittsburgh) Abrams (MET-17)	6,000	ACS	Nucleic acid purine synthesis, rapid tissue growth
7. <u>University of Pennsylvania</u> (Philadelphia) Berkowitz (DRF-134) Blakemore (SG-6) Borei (CP-56) Boyle (C-1959) Breedis (C-1116 C3) Chamberlain (R-18) Chamberlain (C-1984) Dituri (DRF-136) Ehrenstein (C-757 C3) Flexner (C-1807)	Fellow Scholar 6,000 6,147 4,979 7,000 2,612 Fellow 24,624 10,879	DRMF ACS ACS NCI NCI ACS NCI DRMF NCI NCI	Gastric achlorhydria, indicator-exchange resins Pulmonary function, operative proc. & post-op. care Enzyme systems & fertilization Carcin. chem. & normal & ascorbic acid def. guinea pigs Blood supply neoplasms, salamander Microdosimetry determinations radiation therapy Lipid synthesis, particle-free extracts liver Synthesis of steroids Embryonic cell

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		Amount	Agency	Research Subjects
<u>University of Pennsylvania (Cont'd)</u>				
Goddard & Erickson (C-488 C4)		15,000	NCI	Cells, higher plants
Heilbrunn (C-411 C5)		13,876	NCI	Cell division & colloidal change in protoplasm
Jones (CH-2C)	Ext. time	7,700	ACS	Urethane, animal & man
Lucke (#93)		7,700	JCC	Enzyme patterns, metastasis
Lynn (F-176)	Fellow	14,715	ACS	Water-soluble enzy.sys.pigeon liver,fatty acids,acetate
Marshall (C-1957)		50,000	NCI	Protein cytochemistry
Pendergrass (INSTR-64B)		1,500	ACS	Research program
Sayre (C-1822)		5,500	NCI	Relation structure & activity carcinogens
Seibert (BCH-10A)		5,000	ACS	Oncolytic,immunizing,toxic fractions, rat tumor extracts
Williams (C-1618 C)		4,848	NCI	Cytology living thyroid tissue changes
Wilson & Buchanan (C-1135 C3)		10,314	NCI	Nucleic acid metabolism
Wilson & Gurin (IS-4G)		191,694	ACS	Intermediary metabolism protein,fat,carbohydrate by carbon isotopes
8. <u>University of Pittsburgh (Pittsburgh)</u>				
Cox (N-20)		5,000	ACS	Maximum growth, rat
Freiser (C-1882)		7,236	NCI	Trace metals,carcin.subs.interaction nucleic acid metabolites
Hofmann (MET-9B)		6,500	ACS	Unique fatty acid from lactobacillus arabinosus
Mirsky & Harbison (C-1891)		7,776	NCI	Pepsinogen in blood & urine,gastric,human
Olson (N-21)		5,832	ACS	Dietary methyl restriction & leukemia
		32,344		
9. <u>Temple University (Philadelphia)</u>				
Schultz (C-1966)		5,000	NCI	Experimental chloroma
Waldron (C-2008)		2,943	NCI	Abnor.respon.blood coagulation to oral ingestion fat, human
		7,943		
10. <u>Wills Eye Hospital (Philadelphia)</u>				
Leopold (DRIR-254)		4,800	DRMF	Transplants in fungus of eye
11. <u>Wistar Inst. of Anatomy & Biology (Philadelphia)</u>				
Aptekman (C-1646 C)		11,340	NCI	Vaccine
Lewis (C-285 C6)		8,002	NCI	Synthesized compounds & dyes retardation & prevention,rats
Lewis (C-1592 C)		7,560	NCI	Tumor atrophy
		25,902		
<u>RHODE ISLAND</u>				
1. <u>Brown University (Providence)</u>				
Chase (ENV-3B)		1,800	ACS	Skin & its derivatives
Chase (C-592 C2)		7,100	NCI	
Fenton (N-9D)		4,212	ACS	Nutrition with inbred strains, mice
Fenton (C-1995)		2,160	NCI	
Sherman (R-11B)		3,813	ACS	Ionizing radiations on enzyme action of microorganisms
Wilson (INSTR-68A)		10,000	ACS	Histophysiology, histopathology & cytology mouse liver
Wilson (C-510 C5)		14,097	NCI	skin, nutritional requirements,diff.strains mice
		43,182		
2. <u>Providence College (Providence)</u>				
Hickey (MET-22)		7,000	ACS	Labile digitonin precipitable metabolites acetates, chick embryo,living rat liver tissue
3. <u>University of Rhode Island (Kingston)</u>				
Hartung (N-16A)		2,500	ACS	Factors tumor incidence, drosophila melanogaster

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SOUTH CAROLINA

Medical College of South Carolina (Charleston)
 Lynch (C-474 C5)
 Pratt-Thomas (C-1418 C2)

AmountAgencyResearch Subjects

9,650
 8,100
 17,750

NCI
 NCI

Lung tumors
 Effect smegma uterine cervix

TENNESSEE

1. Meharry Medical College (Nashville)
 Hahn
 Johnson (C-1907)

59,872
 7,036
 66,908

AEC
 NCI

Radioactive gold tumor treatment
 Radioactive silver, detection & treatment tumors

2. University of Tennessee (Memphis)

Dulaney (C-624 C4)
 Eades (CS-12B)
 Hardy (CS-14A)
 Overman (H-10B)
 Sprunt (C-1207 C2)
 van Middlesworth (C-1880)
 Whaley (C-1616 C)

11,685
 4,995
 Ext. time
 7,581
 9,500
 5,464
 8,200
 47,425

NCI
 ACS
 ACS
 ACS
 NCI
 NCI
 NCI

Antigenic prop.alpha estradiol protein conjugates
 Amino acid excretion
 Nutrition & alarm response
 Adrenal cortical func., cell membrane, metal ions
 Protein & amino acid levels, Rous sarcoma, chickens
 Thyroid nodule
 Syn. nitrogen isologs chrysene & benzo(a) pyrene, anti-tumor activity

TEXAS

1. Baylor University (Houston)
 Bond (C-1552 C)
 Collins (R-19)
 Hettig (C-1641 R)
 Rose (C-1707 C)
 Spurr (CH-18)

1,512
 8,000
 4,174
 8,963
 4,915
 27,564

NCI
 ACS
 NCI
 NCI
 ACS

Growth, metabolism microorganisms
 Enzymes & radiation sickness, x-ray irradiation intestine
 Hodgkin's disease, nitrogen mustard, x-ray
 Hodgkin's disease, immunology
 Enzyme inhibition, tumor chemotherapeutic agents

2. Odessa College (Odessa)
 Barnes (DRIR-195A)

1,000

DRMF

Sunshine, chemical composition & physiological properties, biochemical compounds

3. St. Joseph's Infirmary (Houston)
 Marcuse (MOR-25)

4,200

ACS

Initial outgrowth, tissue culture, human tumors

4. Southwestern Fdn. for Research & Education (San Antonio)
 Werthessen (C-1609 C)

7,452

NCI

Estronase concentration in blood

5. University of Texas (Austin, Galveston & Houston)

Awapara (MET-8B)
 Awapara (C-1831)
 Barnett (CP-55)
 Clark (INSTR-23F)
 Clark (DRIR-65B)
 Foster (MET-20)
 Hsu (DRF-115A)
 Jirgensons & Awapara (C-1785)
 Oliver (HG-2G)
 Oliver & Lush (G-1)
 Pomerat (CP-12F)
 Reid (BCH-16)
 Rigdon (C-1469 C)
 Wyss (R-17A)

3,500
 9,990
 3,500
 75,000
 15,000
 5,000
 Fellow
 8,253
 6,480
 6,000
 10,000
 5,000
 4,968
 9,000
 161,691

ACS
 NCI
 ACS
 ACS
 DRMF
 ACS
 DRMF
 NCI
 ACS
 ACS
 ACS
 ACS
 NCI
 ACS

Sex hormones, metabolism amino acids, prostate
 ATP & Krebs cycle, cell div., marine eggs
 Growth, hormonal & radiobiologic
 Cobalt-60
 Intermediary metabolism, fungi
 Nuclear cytology human neoplastic tissue
 Blood proteins
 Genetics
 Inheritance patterns, ocular, bovines
 Growth, malignant cells
 Natural metabolic inhibitor
 Skin tumors, methylcholanthrene, white Pekin ducks
 Organic peroxides

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UTAH

1. Utah State Agricultural College (Logan)
Gardner (EG-36A)

Amount	Agency	Research Subjects
2,484	ACS	Genetic & cytological analysis tumorous head, drosophila melanogaster

2. University of Utah (Salt Lake City)
Dougherty (GPB-10E)
Fessas (DRF-138)
Kimmel (F-59C)
Plenk (C-1222 C)
Samuels (EEP-8E)
Samuels & Reich (C-307 C5)
Sandberg (SG-15)

10,314	ACS	Growth lymphoid tissues, mice, inbred
Fellow	DRMF	Chemotherapy of malignancy
Fellow	ACS	Proteinases activated by sulfhydryl
3,000	NCI	Radiation induced leukemia by parabiosis
13,368	ACS	Intermediary metabolism, steroids
15,304	NCI	Steroid metabolism
Scholar	ACS	Metabolism & leukemia
41,986		

VERMONT

- University of Vermont (Burlington)
Novikoff (E-32D)
Pearson (C-431 C5s)

5,000	ACS	Rapid growth, rat liver
13,687	NCI	Enzymatic, biochemical & cytochemical-carcinogenesis
18,687		

VIRGINIA

1. Medical College of Virginia (Richmond)
Clayton (C-17C)
Clayton (C-1541 C)
Clayton (C-1541 C2)
Williams (INSTR-40E)

Ext. time	Agency	Research Subjects
4,500	ACS	Cells, precancerous & tumor-bearing livers
5,184	NCI	Minerals & carcinogenesis
10,000	NCI	
19,684	ACS	Support research beds, radioactive isotopes, chemotherapeutic & endocrine agents, doses

2. University of Virginia (Charlottesville)
Burger (BGH-12)
Chanutin (C-1788)
Chanutin & Hoch (C-269 C4)
Hoch-Ligeti (C-1583 C)
Parson (C-1815)
Rappaport (C-1453 C)

5,886	ACS	Phosphoric acid analogs, nucleotides
12,000	NCI	Iron metabolism, irradiated rats
12,500	NCI	"Trace" components, serum & tissues
7,650	NCI	Nutrition & hepatic tumors
16,048	NCI	Amino acid metabolism
2,000	NCI	Ovular tumors plants
56,084		

WASHINGTON

1. State College of Washington (Pullman)
Nilan & Elliott (R-15B)

2,950	ACS	Oxygen & low temperature & biologic effects x-rays
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2. University of Washington (Seattle)
Dowmont (F-141A)
Everett (C-1681 C)
Fletcher (C-1744 S)
Groman (VI-3)
Hanahan (BGH-4A)
McDonald (CH-13)
Whiteley (CP-46B)

Fellow	ACS	Enzymatic degradation yeast triose phosph. dehydrogenase
5,788	NCI	Tissue localization & metabolism of C ¹⁴
2,260	NCI	Fluorene & aminofluorene derivatives
5,000	ACS	Corynebacterium diphtheriae
6,500	ACS	Phospholipide-splitting enzymes
5,500	ACS	Chemotherapy, prostate
4,968	ACS	Morphogenesis, ciliate protozoa
30,016		

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WISCONSIN

1. Marquette University (Milwaukee)

Laskowski (E-35C)

Saunders (C-1481 C2)

2. University of Wisconsin (Madison)

Baumann (PR-7G)

Cohen (C-822 C3)

Deutsch (C-1786)

Huskins (BO-14E)

Johnson (MET-3C)

Lardy (MET-2C)

LePage (E-13E)

Mahler (BCH-9A)

Miller & Miller (N-5F)

Miller & Miller (C-355 C6)

Miller & Miller (C-355 C7)

Mueller (C-1897)

Parks (F-111B)

Potter (C-646 C4)

Rieck (SG-13)

Riker & Hildebrandt (MOB-26)

Rusch (INSTR-71A)

Rusch & Boutwell (C-828 C4s)

Rusch & Boutwell (CH-15)

Skoog (BO-19D)

Waisman (N-22)

Waisman (C-1792)

Amount

6,500

5,790

12,290

Ext. time

12,500

8,910

4,500

1,836

7,000

4,536

4,500

5,454

12,258

12,258

7,992

Fellow

16,740

Scholar

9,000

25,000

3,780

6,966

7,500

5,292

6,000

162,022

Agency Research Subjects

ACS Nucleolytic enzymes

NCI Melanin pigmentation, higher vertebrates

ACS Diet & tumor formation

NCI Soluble proteins & tissues

NCI Multiple myeloma proteins (Bence-Jones p)

ACS Chromosome reproduction & mitosis

ACS Penicillin

ACS Metabolic regulators

ACS Phosphorylated compounds & cells

ACS DPNH - cytochrome(c) reductase

ACS Mechanism, chemical carcinogenesis

NCI Hepatic neoplasms

NCI Mechanism, action, estrogenic hormones

ACS Enzymes

NCI Biochemical synthesis & growth

ACS Carcinogenic wave-lengths of ultraviolet

ACS Growth & metabolites & basic processes

ACS Genesis, metabolism, chemotherapy neoplas. tissues

NCI Metabolism tumor resistance

ACS

ACS Chem. control growth & organ forma., plant tissue

ACS Antibiotics & metabolic antagonists, growth

NCI Amino acid metabolism, leukemia, rats

Chemotherapy Screening Techniques (1952-53 funds)

150,000

ACS

* Indicates research concerning tobacco

1003537498

NAME OF INDIVIDUAL INVESTIGATOR AND INSTITUTION APPLYING FOR GRANTS FROM TIIC

1. WILEY, Richard H., Chairman, Dept. of Chemistry, Belknap Campus, University of Louisville
2. SEGAL, Maurice S., M.D., Clinical Professor of Medicine, Tufts College Medical School (Boston City Hospital)
3. WASE, A. W., Ph.D., Assistant Prof. of Biological Chemistry, Hahnemann Medical College and Hospital of Philadelphia
4. WENDER, Simon H., Research Professor of Chemistry, University of Oklahoma Research Institute
5. COBE, Herbert M., Professor of Microbiology, Temple University
6. SALTMAN, Paul, D., Ph.D., Assistant Professor, University of Southern California
7. GRIFFIN, A. Clark, Associate Professor of Biochemistry, Stanford University
8. MANN, David E., Jr., Associate Professor of Pharmacology, School of Pharmacy, Temple University
9. GOODSON, Louis H., Ph.D., Senior Research Chemist, Midwest Research Institute
10. AYRE, J. Ernest, M.D., Director, The Cancer Institute at Miami
11. FITZGERALD, P. J., Professor and Executive Head and RICHMOND, Helen, Research Fellow, Department of Pathology, State University of New York College of Medicine
12. FREEDLANDER, B. L., M.D., Director of Cancer Research, Mt. Zion Hospital
13. HOLDEN, Francis R., Senior Physical Chemist, Dept. of Chemistry; VELDEE, M. V., Chairman, Dept. of Biology and RAND, William E., Director of Research, Stanford Research Institute
14. MOTLEY, Hurley Lee, M.D., Professor of Medicine and Director, Cardio-Respiratory Laboratory, University of Southern California School of Medicine
15. WOERNER, Charles Arthur, Ph.D., M.D., Associate Professor of Anatomy, School of Medicine, University of Louisville
16. WELLER, Russell W., M.D., Associate Professor of Pathology, Hahnemann Medical College and Hospital of Philadelphia
17. BAILEY, Paul C., Professor of Biology, Alabama College
18. LOBSTEIN, Otto E., Ph.D., Director of Research, Chem-Tech Laboratories
19. MONTGOMERY, Philip O'Bryan, Associate Professor of Pathology, Southwestern Medical School, The University of Texas
20. STARE, Fredrick J., Ph.D., Professor of Nutrition, and GEYER, Robert P., Ph.D., Assistant Professor of Nutrition, Department of Nutrition, Harvard School of Public Health
21. JACOBS, William Lee, Independent Investigator
22. LIKES, Carl J., Project Supervisor, Virginia Institute for Scientific Research
23. GROSSE, A. V., Director of Project and HAUPTSCHIEIN, Murray, Research Institute of Temple University
24. HAAG, H. B., M.D., Professor of Pharmacology and LARSON, Paul S., Ph.D., Professor of Research Pharmacology, Medical College of Virginia
25. HAWTHORNE, Herbert R., M.D., Chairman, Professor of Surgery, Department of Surgery, Graduate School of Medicine, University of Pennsylvania
26. SHULMAN, Maurice H., M.D., Principal Investigator, LUTZ, Brenton R., and FULTON, George P., Associate Investigators, Department of Biology, Graduate School, Boston University
27. MC KEE, Kelly T., M.D., Associate Professor of Medicine, Medical College of South Carolina
28. MOORE, George E., M.D., Ph.D., and BOCK, Fred, M.S., Roswell Park Memorial Institute
29. HOMBURGER, Freddie, M.D., Director, Cancer Research and Cancer Control Unit, Department of Surgery, Tufts College Medical School

1003537499

March 15, 1955

COMPOSITE LIST OF MEDICAL AND GRADUATE SCHOOLSSUGGESTED FOR SUMMER FELLOWSHIPS

Note: This list includes -

1. Institutions where an SAB member is located
2. Institutions where a TIRC project is located
3. Institutions other than these, suggested by Drs. Lynch, Cattell or Little

<u>Fellowships</u>	<u>Institution</u>	<u>Schools</u>
2	1. University of California	med. and grad.
1	2. University of Southern California	med. or grad.
1	3. Stanford University	medical
1	4. University of Colorado	medical
2	5. Yale University	med. and grad.
1	6. Emory University	med.
1	7. Northwestern University	med. or grad.
2	8. University of Chicago	med. and grad.
2	9. University of Illinois	med. and grad.
1	10. State University of Iowa	medical
1	11. University of Louisville	medical
1	12. Louisiana State University	medical
2	13. Johns Hopkins	med. and grad.
2	14. Harvard	med. and grad.
1	15. Tufts College	medical
2	16. Michigan	med. and grad.
1	17. Minnesota	med. or grad.
1	18. Washington University	med. or grad.
1	19. State University of N. Y. (Brooklyn)	medical
2	20. Columbia University	med. and grad.
2	21. Cornell University	med. and grad.
2	22. New York University	med. and grad.
1	23. University of Rochester	med. or grad.
1	24. Duke University	med. or grad.
1	25. Cincinnati	med. or grad.
1	26. Ohio State University	med. or grad.
1	27. Oregon	med. or grad.
1	28. Hahnemann	medical
1	29. Temple University	med. or grad.
2	30. University of Pennsylvania	med. and grad.
1	31. Medical College of South Carolina	medical
1	32. Vanderbilt	medical
1	33. University of Texas	med. or grad.

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<u>Fellowships</u>	<u>Institution</u>	<u>Schools</u>
1	34. University of Utah	medical
1	35. Medical College of Virginia	medical
1	36. University of Washington	medical
1	37. University of Wisconsin	med. or grad.
<u>1</u>	38. University of North Carolina	medical
49		

Additional suggestions for one more fellowship (R. C. H.)

1	{	University of Maryland	medical
		Bowman Gray	medical
		California Institute of Technology	graduate
		Massachusetts Institute of Technology	graduate

50

TOTAL

1003537501

<u>No.</u>	<u>Investigator</u>	<u>Institution</u>	<u>Subject</u>	<u>Amount</u>	<u>Date</u>	<u>Disposition</u>
64	AYRE, J. Ernest M.D., Director	The Cancer In- stitute at Miami	The chemical determination, both qualitative and quantitative of carcinogenic compounds liberated via smoke and tars, upon the ignition of cigarette paper, tobacco and whole cigarettes; and the physiological effect of these compounds upon human lung tissue grown in tissue culture.	26,200	2/10	
65	GELFANT, Dr. Seymour Assistant Professor of Zoology	Syracuse University	The effect of coal tar derivatives and other constituents of tobacco smoke on cell division <u>in vitro</u> .	16,238	1/31	
66	GRUHZIT, Carl C., M.D. Ph.D., Asso. in Physi- ology & Pharmacology	University of Pennsylvania	Pharmacologic study of nicotine and related alkaloids.	13,915	2/21	
67	WENZEL, Duane G., Asso. Prof. of Pharmacology	University of Kansas, School of Pharmacy	The effect of chronic cigarette smoke inhalation on experimental atherosclerosis of the rabbit.	4,806	2/24	
68	SHERWOOD, Charles E., M.D., Asst. Prof. of Radiology	University of Rochester School of Medicine and Dentistry	Investigation into the natural history of carcinoma of the lung with particular reference to the radiographic appearance of such processes, the earliest manifestation of cancer on chest x-rays and the tabulation of of the relationship of smoking habits and occupation with the incidence of lung cancer.	5,750	2/25	
69	FERGUSON, Frank C., Jr. M.D., Prof. & Chairman of Pharmacology	Albany Medical College	(none on appl.) Effects of tobacco smoke upon the function of the cardiovascular system in animals and man. (RCH)	12,995	3/14	

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March 17, 1955

ACTIVE GRANTS

	<u>Total Value</u>	<u>Date Activated</u>
# 6 - Saltman, Paul D., University of Southern California	\$ 7,776.00	10/1/54
# 8 - Mann, David, Temple University, School of Pharmacy	5,500.00	10/1/54
#24 - Haag, H. B., Medical College of Virginia	33,990.00	11/1/54
#31 - Clarke, Hans T., Columbia University	19,958.00	11/1/54
# 7 - Griffin, A. Clark, M.D. Anderson Hospital, U. of Texas	5,960.00	12/1/54
#28 - Moore, George E., Roswell Park Memorial Institute	30,542.40	12/1/54
#12 - Freedlander, B. L., Mt. Zion Hospital	8,900.00	1/1/55
#20 - Stare, Fredrick J., Harvard School of Public Health	13,613.00	1/1/55
#32 - Cerecedo, Leopold, Fordham University	8,360.00	1/1/55
#42 - Heath, Clark W., Harvard University	15,880.00	1/1/55
# 2 - Segal, Maurice S., Tufts College Medical School	22,000.00	1/1/55
#25 - Hawthorne, Herbert R., University of Pennsylvania, Graduate School of Medicine	30,000.00	2/1/55
#27 - McKee, Kelly T., Medical College of South Carolina	7,900.00	2/1/55
#33 - Sulzberger, Marion B., NYU, Bellevue Medical Center	15,000.00	2/1/55
#50 - Pathologic-Anatomic Project* (Cooperative)	81,000.00	2/1/55
#54 - Barnes, Frederick W., Jr., Johns Hopkins University School of Medicine	11,000.00	2/1/55
#44 - Falk, Hans L., University of Southern California School of Medicine	7,560.00	3/1/55
#52 - Murray, William S., Roscoe B. Jackson Memorial Lab.	43,112.00	3/1/55
#53 - Montgomery, Hugh, University of Pennsylvania, Medical School	10,667.50	3/1/55
#14 - Motley, Hurley Lee, University of Southern California	21,000.00	6/1/55
#51 - Pratt-Thomas, H. R., Medical College of South Carolina	8,134.50	7/1/55
#66 - Gruhzt, Carl C., University of Pennsylvania, Graduate School of Medicine	<u>13,915.00</u>	7/1/55
<u>TOTAL</u>	\$421,768.40	

* List of Pathologic-Anatomic Project participants dated February 21, 1955 was sent to members of the SAB on February 28, 1955.

1003537503

Renewals of Active Grants Approved through January, 1956 (continued)

- #53R1 - Hugh Montgomery, M.D., Associate Professor of Medicine, University of Pennsylvania, Medical School, Philadelphia 4, Pa. "Influence of Tobacco Smoking on the Blood Flow of Skin and of Muscles of Extremities in Sympathectomized and Unsympathectomized Subjects."
\$10,043.75 - One year
- #62R1 - Joseph H. Hafkenschiel, M.D., Director of Cardiopulmonary Unit, Lankenau Hospital, Overbrook, Philadelphia 31, Pennsylvania. "Measurement of Coronary Blood Flow, Cardiac Work and Cardiac Oxygen and Carbohydrate Metabolism in Normotensive Subjects Before and After Intravenous Nicotine and After Smoking Standard Cigarettes."
\$7,938.00 - One year

Renewals of Active Grants Approved through May, 1956

- #91R1 - Richard J. Bing, M.D., Professor of Medicine, Washington University, St. Louis, and Chief of the Washington University Medical Service, Veterans Administration Hospital, St. Louis, Missouri. "I. The Effect of Nicotine on Purified Actomyosin Bands. II. The Effect of Tobacco Smoking on the Coronary Circulation and Myocardial Metabolism of Individuals with Coronary Arteriosclerosis."
\$9,790.00 - One year
- #66R1 - Carl C. Gruhzt, Ph.D., M.D., Associate in Physiology and Pharmacology, Department of Physiology and Pharmacology, Graduate School of Medicine, University of Pennsylvania, Philadelphia 4, Pa. "Pharmacologic Study of Nicotine and Related Alkaloids."
\$13,915.00 - One year
- #74R1 - Cecilie Leuchtenberger, Ph.D., Associate Professor of Cytology and Biology, Institute of Pathology, Western Reserve University, Cleveland 6, Ohio. "Quantitative Analysis of Nucleoproteins in Tissues from Animals Subjected to Tobacco Smoke by Microspectrophotometry and Interference Microscopy Correlated with Cytological and Histological Studies."
\$17,282.20 - One year
- #14R1 - Hurley Lee Motley, M.D., Professor of Medicine and Director Cardio-Respiratory Laboratory, University of Southern California School of Medicine, 3518 University Avenue, Los Angeles 7, California. "A Study of the Effects of Smoking on Pulmonary Function."
\$19,000.00 - One year
- #51R1 - H. R. Pratt-Thomas, M.D., Professor of Pathology, Medical College of South Carolina, 16 Lucas Street, Charleston, South Carolina. "Application of a New Bio-Assay Technique in Examination of Cigarette Smoke Condensate for Possible Carcinogens."
\$8,634.50 - One year

1003537504

Renewals of Active Grants Approved through May, 1956

- #72R1 - R. H. Rigdon, M.D., Professor of Pathology and Director, Laboratory of Experimental Pathology, University of Texas Medical Branch, Galveston, Texas. "(a) Study Effect of Methylcholanthrene on Tissues of the Duck. (b) Compare Reaction in Chicken and Turkey. (c) Complete Study of the Effect of Methylcholanthrene on Trachea. (d) Culture Tumor in Yolk Sac of Developing Chick Embryos and on Chorio-Allantoic Membrane of Chicks." \$5,290.00 - One year
- #68R1 - Charles E. Sherwood, M.D., Assistant Professor of Radiology, The University of Rochester School of Medicine and Dentistry, 260 Crittenden Boulevard, Rochester, New York. "Investigation into the Natural History of Carcinoma of the Lung with Particular Reference to the Radiographic Appearance of Such Processes, the Earliest Manifestation of Cancer on Chest X-Rays and the Tabulation of the Relationship of Smoking Habits and Occupation with the Incidence of Lung Cancer." \$5,750.00 - One year
- #85R1 - Sam Sorof, Ph.D., Associate Member, The Institute for Cancer Research and the Lankenau Hospital Research Institute, 7701 Burholme Avenue, Fox Chase, Philadelphia 11, Pennsylvania. "Chemical and Physical Studies on the Tissue Proteins Involved in Chemical Carcinogenesis." \$2,160.00 - One year (Supplement)
- #89R1 - Caroline Bedell Thomas, M.D., Associate Professor of Medicine, The Johns Hopkins University School of Medicine, 710 North Washington Street, Baltimore 5, Maryland. "The Significance of Individual Smoking Patterns in Healthy Young Adults. (a) Comparison of Smokers and Non-Smokers in Regard to 1) Family History of Hypertension and/or Coronary Artery Disease; 2) Physiologic Characteristics; and 3) Psychologic Traits. (b) Ballistocardiographic Studies of the Circulatory Response to Smoking, with Analysis of Differences Associated with Family History, Physiologic and Psychologic Traits." \$8,510.00 - One year
- #92R1 - Janet Travell, M.D., Associate Professor, Clinical Pharmacology, Cornell University Medical College, 1300 York Avenue, New York 21, N. Y. "Cardiac Effects of Nicotine in the Rabbit with Experimental Coronary Atherosclerosis." \$11,000.00 - One year
- #97R1 - J. Edwin Wood, M.D., Massachusetts Memorial Hospitals, Evans Memorial, 65 East Newton Street, Boston 18, Massachusetts and Instructor in Medicine, Boston University School of Medicine. "The Acute Effect of Inhalation of Tobacco Smoke Upon Reactive Hyperemia Blood Flow of the Foot in Normal Individuals and Patients with Peripheral Vascular Disease." \$4,000.00 - One year

1003537505

Grants Approved January 1956

- #117 - Victor Richards, M.D., Professor of Surgery, Executive Head of Department of Surgery, Stanford University School of Medicine, Sacramento and Webster Streets, San Francisco 15, California. "A Comparative Study of the Effects of Whole and Fractionated Extracts of Cigarette Smoke and Those of Known Carcinogens on I. The Cytology and Nuclear DNA Content of Epidermis in Various Strains of Mice and/or II. The Cytology and Nuclear DNA Content of Lung and Epithelium of the Bronchial Tree of Mice and Hamsters." \$33,800.00 - One year

Grants Approved May 1956

- #126 - James Bonner, Ph.D., Professor of Biology, California Institute of Technology, 1201 East California Street, Pasadena, California. "Enzymatic Study of Methylation Reactions in Plant Tissue." \$9,680.00 - One year
- #128 - Paul S. Larson, Ph.D., Professor of Pharmacology, Medical College of Virginia, Richmond 19, Virginia. "Enzymatic Transformations of Nicotine." \$29,080.00 - One year
- #134 - Tissue Culture
- (a) Contribution to Tissue Culture Association- \$5,000
One year
 - (b) George O. Gey, M.D., Johns Hopkins University.
Fellowship for Study of the Culture of Human
Lung Tissue in Vitro - One year \$8,000
- #123 - Jerry Hart Jacobson, M.D., Director, Division of Electrophysiology, New York Eye and Ear Infirmary. "A Comparison of Electroretinography as a Means of Evaluating the Effect of Vasoconstrictor Drugs Upon Cerebral and Retinal Circulation to Other Techniques for this Determination." \$4,200.00 - One year

1003537506

Renewals of Active Grants Approved through August, 1956

- #93R1 - Richard L. Wechsler, M.D., Clinical Physiologist, Montefiore Hospital Institute of Research, 3459 Fifth Avenue, Pittsburgh 13, Pennsylvania. "Effect of Cigarette Smoking and Intravenous Nicotine on Cerebral Blood Flow, Cerebral Metabolism, Blood Gases, Blood pH, Arterial Pulse Pressure Curves, Electrocardiograms, and Electroencephalograms in People of the Older Age Group with Arteriosclerosis."
\$10,000.00 - One year
- #70R1 - Jack Freund, M.D., Lecturer in Pharmacology, Medical College of Virginia, Richmond 19, Virginia. "Further Investigation of the Physiological Effects of Sham and Actual Smoking on the Peripheral Circulation in the Normal Individual."
\$8,718.00 - Six months
- #89R1 - Caroline Bedell Thomas, M.D., Department of Preventive Medicine, The Johns Hopkins University School of Medicine, 700 North Wolfe Street, Baltimore 5, Maryland. "The Significance of Individual Smoking Patterns in Healthy Young Adults."
\$2,000.00 - One year (Supplement to grant approved in May)
- #29BR1 - F. Homburger, M.D., Director of Cancer Research and Cancer Control Unit, New England Center Hospital, 30 Bennet Street, Boston, Massachusetts. "A Study on the Effects of Various Components of Tobacco and Cigarette Paper upon the Behavior of Transplantable Tumors."
\$25,760.00 - One year
- #12R2 - B. L. Freedlander, M.D., Director of Cancer Research, Mount Zion Hospital, 1600 Divisadero Street, San Francisco 15, California. "Experiments on the Carcinogenic and Co-carcinogenic Action of Tobacco Products."
\$11,800.00 - One year
- #85R1 - Sam Sorof, Ph.D., Associate Member, The Institute for Cancer Research and Lankenau Hospital Research Institute, 7701 Burholme Avenue, Fox Chase, Philadelphia 11, Pa. "Chemical and Physical Studies on the Tissue Proteins Involved in Chemical Carcinogenesis."
\$18,855.00 - One year
- #79R1 - Joseph B. Kirsner, M.D., Professor of Medicine, University of Chicago, Department of Medicine, 950 East 59th Street, Chicago 37, Illinois. "Effect of Tobacco Smoking upon Basal Gastric Secretion in Man - Part II."
\$9,188.00 - One year
- #24R1 - Harvey B. Haag, M.D., Professor of Pharmacology, Medical College of Virginia, Richmond 19, Virginia. "Preparation for Publication of a Book on the Biologic Aspects of Tobacco and its Smoke."
\$3,274.40 - Two months
- #31R2 - G. Y. Gottschall, Ph.D. (Hans T. Clarke) Assistant Professor of Biochemistry, Columbia University, College of Physicians and Surgeons, 630 West 168th Street, New York. "Biochemistry of White Blood Cells."
\$16,458.00 - One year

1003537507

Renewals of Active Grants Approved through August, 1956 - Cont'd.

- #104R1 - Josef Brozek, Ph.D., Professor, Laboratory of Physiological Hygiene, University of Minnesota, Stadium, Gate 27, Minneapolis 14, Minnesota. "Biological Characteristics of Men and Their Smoking Habits." \$15,000.00 - One year
- #84R1 - Isaac Schour, D.D.S., Ph.D., D.Sc., Professor of Histology and Embryology and Dean, University of Illinois College of Dentistry, Chicago 12, Illinois. "Histologic Changes in the Oral, Pharyngeal and Nasal Tissues of Experimental Animals Subjected to Tobacco Smoke." \$17,687.69 - One year

Renewals of Active Grants Approved through December, 1956

- #116R1 - Ancel Keys, Ph.D., Director, Laboratory of Physiological Hygiene, University of Minnesota, Stadium, Gate 27, Minneapolis 14, Minnesota. "Comparative Epidemiology of Smoking and of the Incidence of Heart Disease." \$8,800 - One year
- #44R2 - Hans L. Falk, Ph.D., Assistant Professor of Biochemistry, University of Southern California School of Medicine, 1200 North State Street, Los Angeles 33, California. "The Experimental Production of Hydrocarbons in Simulated Cigarette Smoking - Their Analysis and Quantitation." \$11,385.00 - One year
- #54R2 - Frederick W. Barnes, Jr., M.D., Associate Professor of Medicine, The Johns Hopkins University School of Medicine, 710 North Washington Street, Baltimore 5, Maryland. "Restorative Response of Tissues - Circulation of Tissue Proteins in Serum Globulins." \$9,500.00 - One year
- #32R2 - Leopold R. Cerecedo, Ph.D., Professor of Biochemistry, Department of Chemistry, Fordham University, New York 58, New York. "A Study of Early Chemical Changes in the Lungs of Tumor-bearing Rats and Mice." \$10,925.00 - One year
- #117R1 - Victor Richards, M.D., Professor of Surgery, Stanford University School of Medicine, Clay and Webster Streets, San Francisco 15, California. "A Comparative Study of the Effects of Whole and Fractionated Extracts of Cigarette Smoke and Those of Known Carcinogens on I. The Cytology and Nuclear DNA Content of Epidermis in Various Strains of Mice." \$19,000 - One year
- #52R2 - William S. Murray, Sc.D., Administrative Director, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. "The Production of Genetically Controlled Animals and Tumors for Possible Use by TIIC Grantees in Experimental Research on Tobacco in Relation to Health by (a) The Expansion of Known Inbred Stocks and Sources of Tumor Supply; (b) The Production of Such Hybrids or Heterozygous Types as Becomes Necessary; and (c) The Relation of this Material to Specific Experimental Work at the Laboratory." \$32,359.00 - One year

1003537508

Renewals of Active Grants Approved through December, 1956 - Cont'd.

#4AR1 - Simon H. Wender, Ph.D., Research Professor of Chemistry, University of Oklahoma - Research Institute, Norman, Oklahoma. "Qualitative and Quantitative Studies of the Individual Polyphenol Content of Cigarette Tobacco and of the Smoke and "Tars" Resulting from Cigarette Smoking; and also to Study the Fate of these Compounds in the Animal Respiratory System."
\$8,970.00 - One year

#42R2 - Charles McArthur, Ph.D. (Heath-Farnsworth) Psychologist to the University Health Services, Harvard University, 78 Mt. Auburn Street, Cambridge, Massachusetts. "Monograph on Social and Personal Determinants of Smoking Behavior."
\$7,500.00 - Six months

#25R2 - Herbert R. Hawthorne, M.D., Chairman, Department of Surgery, Graduate School of Medicine, University of Pennsylvania, 19th & Lombard Streets, Philadelphia 46, Pennsylvania. "The Attempted Production of Pulmonary Neoplasms in Experimental Animals by Exposure of the Tracheo-Bronchial System to Tobacco Smoke."
\$30,000.00 - One year

#50R2 - Pathologic-Anatomic Study of Cellular Changes in Human Lungs.
\$32,026.00 - Six months

A. Russell W. Weller, M.D., Ephrata Community Hospital, Ephrata, Pa. \$4,148.00

C. Kenneth P. Knudtson, M.D., University of Washington, Seattle, Wash. \$2,502.00

D. Russell S. Fisher, M.D., University of Maryland, Baltimore 2, Md. \$3,542.00

E. D. Murray Angevine, M.D., The University of Wisconsin, Madison 6, Wisc. \$3,021.00

G. E. M. Butt, M.D., Los Angeles County General Hospital, Los Angeles 33, Calif. \$3,047.00

H. Russell L. Holman, M.D., Louisiana State University, New Orleans 12, La. \$4,253.00

I. Thomas C. Laipply, M.D., Northwestern University Medical School, Chicago 11, Ill. \$2,387.00

J. H. R. Pratt-Thomas, M.D., Medical College of South Carolina, Charleston, S.C. \$2,867.00

* K. William H. Carnes, M.D., Stanford University School of Medicine, San Francisco 15, Calif. \$2,781.00

L. Marvin Kuschner, M.D., New York University-Bellevue Medical Center, New York 16, N. Y. \$3,478.00

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WASHINGTON, D.C. 20540

1003537510

Grants Approved January 1956

- #117 - Victor Richards, M.D., Professor of Surgery, Executive Head of Department of Surgery, Stanford University School of Medicine, Sacramento and Webster Streets, San Francisco 15, California. "A Comparative Study of the Effects of Whole and Fractionated Extracts of Cigarette Smoke and Those of Known Carcinogens on I. The Cytology and Nuclear DNA Content of Epidermis in Various Strains of Mice and/or II. The Cytology and Nuclear DNA Content of Lung and Epithelium of the Bronchial Tree of Mice and Hamsters."
\$33,800.00 - One year

Grants Approved May 1956

- #126 - James Bonner, Ph.D., Professor of Biology, California Institute of Technology, 1201 East California Street, Pasadena, California. "Enzymatic Study of Methylation Reactions in Plant Tissue."
\$9,680.00 - One year
- #128 - Paul S. Larson, Ph.D., Professor of Pharmacology, Medical College of Virginia, Richmond 19, Virginia. "Enzymatic Transformations of Nicotine."
\$29,080.00 - One year

#134 - Tissue Culture

- (a) Contribution to Tissue Culture Association \$5,000
One year
- (b) George O. Gey, M.D., Johns Hopkins University.
Fellowship for Study of the Culture of Human
Lung Tissue in Vitro - One year \$8,000

- #123 - Jerry Hart Jacobson, M.D., Director, Division of Electrophysiology, New York Eye and Ear Infirmary. "A Comparison of Electroretinography as a Means of Evaluating the Effect of Vasoconstrictor Drugs Upon Cerebral and Retinal Circulation to Other Techniques for this Determination."
\$4,200.00 - One year

- #85 - Sam Sorof, Ph.D., Associate Member, The Institute for Cancer Research and the Lankenau Hospital Research Institute, 7701 Burholme Avenue, Fox Chase, Philadelphia 11, Pennsylvania. "Chemical and Physical Studies on the Tissue Proteins Involved in Chemical Carcinogenesis."
\$2,160.00 - Supplement to original grant.

Grants Approved August 1956

- #117 - Victor Richards, M.D., Professor of Surgery, Stanford University School of Medicine, Sacramento & Webster Streets, San Francisco 15, California.
\$800.00 - Supplement to grant approved January, 1956. To be used for purchase of a new Frieden calculator.

1003537511

Grants Approved August 1956 - Cont'd.

#134 - Tissue Culture

- (c) Charles M. Pomerat, Ph.D., Professor of Cytology,
The University of Texas - Medical Branch.
Fellowship for Tissue Culture Studies of Human
Lung. \$7,000

- #86A - Sydney C. Rittenberg, Ph.D., Professor of Bacteriology, University
of Southern California, University Park, Los Angeles 7, California.
"Studies on the Mechanism of Bacterial Metabolism of Nicotine and
Related Compounds."
\$4,104.00 - One year

Grants Approved December 1956

- #138 - Morton I. Grossman, Ph.D., M.D., Associate Clinical Professor of
Medicine, University of California Medical Center, Los Angeles 24,
California. "The Effect of Smoking on Certain Gastric Functions."
\$8,400.00 - One year
- #142 - Paul D. Saltman, Ph.D., Assistant Professor, Department of Biochemistry
and Nutrition, University of Southern California, University Park,
Los Angeles 7, California. "Some Aspects of Amino Acid Metabolism
in Tobacco Leaves."
\$7,560.00 - One year
- #146 - Hugh Montgomery, M.D., Associate Professor of Medicine, Hospital
of the University of Pennsylvania - Vascular Section, 3400 Spruce
Street, Philadelphia, Pennsylvania. "Influence of Nicotine (i.v.)
and Tobacco Smoking on Blood Flow in Human Skin and Skeletal Muscle."
\$10,500.00 - One year
- #137 - Samuel Bellet, M.D., Director, Division of Cardiology, Philadelphia
General Hospital - Research Fund, 34th Street and Curie Avenue,
Philadelphia 4, Pennsylvania. "Cardiovascular Effects of Smoking
and of Nicotine in Experimental Animals and in Human Subjects."
\$10,400.00 - One year

1003537512

Renewals of Active Grants Approved March 1957

- #24 - Harvey B. Haag, M.D., Professor of Pharmacology, Medical College of
#24R1 - Virginia, Richmond 19, Virginia. "Preparation for Publication of a
Book on the Biologic Aspects of Tobacco and its Smoke."
Supplement of \$4,911.60 - Three months
\$15,630.00 - Seventeen months
- #32R2 - Leopold R. Cerecedo, Ph.D., Professor of Biochemistry, Department of
Chemistry, Fordham University, New York 58, N.Y. "A Study of Early
Chemical Changes in the Lungs of Tumor-Bearing Rats and Mice."
\$10,925.00 - One year
- #70R1 - Jack Freund, M.D., Associate in Medicine, Medical College of Virginia,
Richmond 19, Virginia. "Investigation of the Physiological Effects of
Sham and Actual Smoking on the Peripheral Circulation in the Normal
Individual."
\$4,359.00 - Pro Rata Extension, Three months
- #80R2 - Fellowship Program
\$35,300.00 - 1957
- #91R2 - Richard J. Bing, M.D., Professor of Medicine, Veterans Administration
Hospital, 915 North Grand Boulevard, St. Louis 6, Missouri. "The
Effect of Smoking on the Coronary Blood Flow and Certain Phases of
Myocardial Metabolism in Patients with Arteriosclerotic or Hypertensive
Cardiovascular Disease."
\$9,904.00 - One year
- #128R1 - Paul S. Larson, Ph.D., Professor of Pharmacology, Medical College of
Virginia, Richmond 19, Virginia. "Enzymatic Transformations of
Nicotine."
\$23,142.90 - One year

Renewals of Active Grants Approved May 1957

- #62R1 - Joseph H. Hafkenschiel, M.D., Director, Cardiopulmonary Unit, The
Lankenau Hospital, Lancaster & City Line Avenues, Philadelphia 31,
Pa. "Measurement of Coronary Blood Flow, Cardiac Work and Cardiac
Oxygen and Carbohydrate Metabolism in Normotensive Subjects Before and
After Intravenous Nicotine and After Smoking Standard Cigarettes."
\$3,969.00 - Six months
- #51R2 - H. R. Pratt-Thomas, M.D. Professor of Pathology, Medical College of
South Carolina, 16 Lucas Street, Charleston 16, S.C. "Application of
a New Bio-Assay Technique in Examination of Cigarette Smoke Condensate
for Possible Carcinogens."
\$8,310.50 - One year
- #92R2 - Janet Travell, M.D., Associate Professor, Clinical Pharmacology,
Department of Pharmacology, Cornell University Medical College, 1300
York Avenue, New York 21, N.Y. "Cardiac Effects of Nicotine in the
Rabbit with Experimental Coronary Atherosclerosis."
\$16,170.00 - One year

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Grants Approved March 1957

- #141 - Alvin I. Kosak, Ph.D., Associate Professor of Chemistry, New York University, Washington Square College, New York, N.Y. "The Isolation and Identification of Certain Lower-Boiling Components of Cigarette Smoke."
\$6,038.00 - One year
- #148 - David L. Simon, M.D., Department of Internal Medicine, Cincinnati General Hospital, Cincinnati 29, O. "The Effects of Pipe Smoking and Cigar Smoking on the Cardiovascular System of Man."
\$2,940.00 - One year
- #150 - John W. Eckstein, M.D., Assistant Professor, Department of Internal Medicine, College of Medicine, State University of Iowa, Iowa City, Ia. "Responses of the Peripheral Veins in Man to the Intravenous Administration of Nicotine."
\$3,795.00 - One year
- #152 - Charles B. McCants, Ph.D., Department of Soils, School of Agriculture, North Carolina State College, Raleigh, N.C. "Arsenic Content of Soils and Absorption by the Tobacco Plant."
\$3,000.00 - One year
- #153 - Jack Freund, M.D., Associate in Medicine, Medical College of Virginia, Richmond 19, Virginia. "A Study of the Effects of Cigarette Smoking on the Peripheral Circulation of Individuals with Arteriosclerosis Obliterans and Other Peripheral Vascular Diseases, Utilizing Multi-technical Procedures."
\$7,981.00 - Nine months
- #154 - Fred G. Bock, M.S., Senior Cancer Research Scientist, Health Research, Inc., Roswell Park Division, 666 Elm St., Buffalo 3, N.Y. "Investigation of the Biological Effects of Cigarette Smoke." (See #28 and #28R1 - George E. Moore, M.D.)
\$14,525.00 - One year

Grants Approved May 1957

- #155 - Duane G. Wenzel, Ph.D. Professor of Pharmacology, The School of Pharmacy, University of Kansas, Lawrence, Kansas. "The Determination of the Chronic Effects of Orally Administered Nicotine on Serum Cholesterol and Phospholipids; the Electrocardiographic Response to Ergonovine; and the Vascular Pathology of Cholesterol-fed Rabbits."
\$6,944.00 - One year
- #157 - R. H. Rigdon, M.D., Professor of Pathology, University of Texas - Medical Branch, Galveston, Texas. "Effect of Tobacco Tar on Respiratory Tract of the Duck."
\$5,278.50 - One year

1003537514

Grants Approved May 1957 - Cont'd.

- #158 - Tom G. Bowery, Ph.D., Pesticide Residue Laboratory, Chemistry Department, North Carolina State College, Raleigh, N.C. "TDE and Endrin Residues in Cigarette Smoke."
\$9,000.00 - One year
- #159 - Cecilie Leuchtenberger, Ph.D., Associate Professor of Cytology and Biology, Institute of Pathology, Western Reserve University, 2085 Adelbert Road, Cleveland 6, O. "A Correlated Histological, Cytological and Cytochemical Study of the Tracheo-bronchial Tree from Mice Exposed to Cigarette Smoke."
\$22,928.12 - One year

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Grants Approved May 1957 - Cont'd.

- #158 - Tom G. Bowery, Ph.D., Pesticide Residue Laboratory, Chemistry Department, North Carolina State College, Raleigh, North Carolina. "TDE and Endrin Residues in Cigarette Smoke."
\$9,000.00 - One year
- #159 - Cecilie Leuchtenberger, Ph.D., Associate Professor of Cytology and Biology, Institute of Pathology, Western Reserve University, Cleveland 6, Ohio. "A Correlated Histological, Cytological and Cytochemical Study of the Tracheo-bronchial Tree from Mice Exposed to Cigarette Smoke."
\$22,928.12 - One year

Grants Approved August 1957

- #160 - Marion B. Sulzberger, M.D., Professor and Chairman, Department of Dermatology and Syphilology, NYU-Post-Graduate Medical School, New York 16, N.Y. "Investigation of the Effects of Tobacco on the Human Vascular System in Healthy Volunteers as well as in Patients with Occlusive Vascular Disease; Particular Attention to be Directed at the Possibility that Certain Tobacco Effects are Based on Peculiar Allergic Susceptibility of Specific Individuals Rather than Upon Obligatorily Toxic Products in Tobacco Smoke. Also, the Possible Influence of Habitual Smoking Upon Vascular Responses is to be Ascertained."
\$15,000.00 - One year
- #164 - R. F. Dawson, Ph.D., Department of Botany, Columbia University, New York 27, New York. "An Investigation of the Metabolism of Pyridine Compounds in the Tobacco Plant."
\$8,400.00 - Three years
- #165 - E. D. Warner, M.D., Professor of Pathology, The State University of Iowa College of Medicine, Iowa City, Iowa. "Correlation of Bronchial Epithelial Changes with Comparable Changes in Other Organs - A Pathologic-Anatomic Study."
\$1,000.00 - One year
- #168 - Caroline Bedell Thomas, M.D., Associate Professor of Medicine, The Johns Hopkins School of Medicine, Baltimore 5, Maryland. "Studies on Smoking in Healthy Young Adults."
\$10,810.00 - One year
- #171 - E. M. Butt, M.D., Chief Pathologist, Los Angeles County General Hospital, Los Angeles 33, California. "Geographical Study of Trace Metal Patterns in Pulmonary Tissue."
\$5,000.00 - One year

Grants Approved November 1957

- #174 - Russell L. Holman, M.D., Professor and Head, Department of Pathology, Louisiana State University School of Medicine, New Orleans 12, Louisiana. "The Influence of Tobacco Smoking on Acute Myocardial Infarction."
\$7,705.00 - One year

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Grants Approved November 1957 - Cont'd.

- #170 - Edward W. Pelikan, M.D., Associate Professor, Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston 18, Massachusetts. "A Study of Structure-Activity Relationships Among Drugs Which Affect Nicotine-Sensitive Physiological Mechanisms."
\$10,465.00 - One year
- #181 - Benjamin A. Rubin, Ph.D., Assistant Professor, Department of Public Health and Preventive Medicine, Baylor University College of Medicine, Houston 25, Texas. "An Evaluation of the Phenomenon of Tumor Growth Enhancement as an Assay for Carcinogens Among the Polycyclic Hydrocarbons and Related Compounds."
\$5,175.00 - One year
- #134C- Donald M. Pace, Ph.D., Professor of Physiology and Director, Institute for Cellular Research, University of Nebraska, Lincoln, Nebraska. "To Study the Effects of Some of the Constituents of Tobacco Smoke on Various Strains of Tissue Cells Cultivated In Vitro."
\$7,200.00 - One year

1003537518

Renewals of Active Grants Approved August 1957

- #4AR2 - Simon H. Wender, Ph.D., Research Professor of Chemistry, University of Oklahoma - Research Institute, Norman, Oklahoma. "Qualitative and Quantitative Studies of the Individual Polyphenol Content of Cigarette Tobacco and of the Smoke and "Tars" Resulting from Cigarette Smoking; and also to Study the Fate of These Compounds in the Animal Respiratory System."
\$15,400.00 - One year
- #14R2 - Hurley L. Motley, M.D., Professor of Medicine and Director of Cardio-Respiratory Laboratory, University of Southern California, 1212 Shatto Street, Los Angeles 17, California. "A Study of the Effects of Smoking on Pulmonary Function."
\$15,000.00 - One year
- #86AR1 - Sydney C. Rittenberg, Ph.D., Professor of Bacteriology, University of Southern California, University Park, Los Angeles 7, California. "Studies on the Mechanism of Bacterial Metabolism of Nicotine and Related Compounds. The Ultimate Goal of the Project is the Elucidation of the Intermediary Metabolism of Nicotine Oxidation."
\$7,344.00 - One year
- #126R1 - James F. Bonner, Ph.D., Professor of Biology, California Institute of Technology, 1201 East California Street, Pasadena, California. "Enzymatic Study of Methylation Reactions in Plant Tissue."
\$9,717.00 - One year
- #134AR1 - Tissue Culture Fellowship - George O. Gey, M.D., Director, Finney-Howell Cancer Research Laboratory, Department of Surgery, The Johns Hopkins Hospital, Baltimore 5, Maryland. "For Studying the Culture of Human Lung Tissue and the Effects of Known and Possible Carcinogenic Agents Upon Such Tissue."
\$15,000.00 - One year

Renewals of Active Grants Approved November, 1957

- #52R3 - William S. Murray, Sc.D., Senior Staff Scientist and Associate Director, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. "The Production of Genetically Controlled Animals and Tumors for Possible Use by T.I.R.C. Grantees in Experimental Research on Tobacco in Relation to Health by (a) The Expansion of Known Inbred Stocks and Sources of Tumor Supply; (b) The Production of Such Hybrids or Heterozygous Types as Becomes Necessary; and (c) The Relation of this Material to Specific Experimental Work at the Laboratory."
\$33,108.00 - One year
- #54R3 - Frederick W. Barnes, Jr., M.D., Ph.D., Associate Professor of Medicine and of Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore 5, Maryland. "The Role of Hyperplasia in Tissue Response to Chronic Damage."
\$10,116.00 - One year

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Renewals of Active Grants Approved November 1957 - Cont'd.

- #134BR1 - C. M. Pomerat, Ph.D., Professor of Cytology, The University of Texas - Medical Branch, Galveston, Texas. "Tissue Culture Studies of Human Lung."
\$12,020.00 - One year
- #142R1 - Paul D. Saltman, Ph.D., Assistant Professor, Department of Biochemistry and Nutrition, University of Southern California, Los Angeles 7, California. "Some Aspects of Amino Acid Metabolism in Tobacco Leaves."
\$7,560.00 - One year
- #137R1 - Samuel Bellet, M.D., Director, Division of Cardiology, Philadelphia General Hospital, Philadelphia 4, Pennsylvania. "Cardiovascular Effects of Smoking and of Nicotine in Experimental Animals and in Human Subjects."
\$7,770.00 - One year
- #12R2 - B. L. Freedlander, M.D., Director of Cancer Research, Mount Zion Hospital, San Francisco 15, California. "Experiments on the Carcinogenic and Cocarcinogenic Action of Tobacco Products."
\$2,000.00 - Pro rata extension to March 1, 1958
- #50R2 - Pathologic-Anatomic Study of Cellular Changes in Human Lungs.
\$1,500.00 - Supplement. For further study of data obtained under the original P.A.S. grant.
- #80R3 - Fellowship Program for 1958
\$36,900.00. This amount includes balance of \$1,900.00 from last year's program. - One year
- #29BR2 - F. Homburger, M.D., President, Bio-Research Institute, Inc., Cambridge 41, Massachusetts. "A Study on the Effects of Various Components of Tobacco and Cigarette Paper Upon the Behavior of Transplantable Tumors."
\$25,760.00 - One year

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Grants Approved November 1957 - Cont'd.

- #170 - Edward W. Pelikan, M.D., Associate Professor, Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston 18, Mass. "A Study of Structure-Activity Relationships Among Drugs Which Affect Nicotine-Sensitive Physiological Mechanisms."
\$10,465.00 - One year
- #181 - Benjamin A. Rubin, Ph.D., Assistant Professor, Department of Public Health and Preventive Medicine, Baylor University College of Medicine, Houston 25, Texas. "An Evaluation of the Phenomenon of Tumor Growth Enhancement as an Assay for Carcinogens Among the Polycyclic Hydrocarbons and Related Compounds."
\$5,175.00 - One year
- #134C- Donald M. Pace, Ph.D., Professor of Physiology and Director, Institute for Cellular Research, University of Nebraska, Lincoln, Nebraska. "To Study the Effects of Some of the Constituents of Tobacco Smoke on Various Strains of Tissue Cells Cultivated In Vitro."
\$7,200.00 - One year

Grants Approved February 1958

- #141 - Alvin I. Kosak, Ph.D., Associate Professor of Chemistry, New York University, Washington Square College, New York, N.Y. "The Isolation and Identification of Certain Lower-Boiling Components of Cigarette Smoke."
\$410.00 - Supplement to original grant. To be used for purchase of cigarettes.
- #180 - B. L. Freedlander, M.D., Director of Cancer Research, Mount Zion Hospital, San Francisco 15, California. "Experiments on the Carcinogenic and Co-carcinogenic Action of Tobacco Products."
\$16,125.00 - One year (This amount includes \$2,000.00 pro rata extension approved November 1957. See grant #12R2.)
- #185 - Hans L. Falk, Ph.D., Assistant Professor of Biochemistry, University of Southern California School of Medicine, Los Angeles 33, California. "A Compilation of Fluorescence Spectra of Polycyclic Aromatic Hydrocarbons and Closely Related Compounds Which Are of Interest in the Study of Air Pollutants, and Cigarette Smoke in Relation to Lung Cancer Etiology."
\$7,475.00 - Fifty weeks

Grants Approved May 1958

- #197 - Richard U. Byerrum, Ph.D., Professor of Chemistry, Michigan State University, East Lansing, Michigan. "Biosynthesis of the Pyridine Ring of Nicotine."
\$6,984.00 - One year

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Grants Approved May 1958 - Cont'd.

- #198 - Maurice S. Segal, M.D., Clinical Professor of Medicine, Tufts University School of Medicine, Boston, Mass. "Relationship of Cigarette Smoking to Chronic (Obstructive) Pulmonary Emphysema."
\$5,000.00 - One year
- #203 - Caroline Bedell Thomas, M.D., Associate Professor of Medicine, The Johns Hopkins School of Medicine, Baltimore 5, Maryland.
"a. Studies of Genetic Differences Between Smokers and Non-smokers. b. Studies of Psychological Differences Between Smokers and Nonsmokers As Shown By Comparison of Figure Drawings."
\$11,500.00 - One year

1003537522

#197 - Richard W. Ferguson, Ph.D., Professor of Chemistry, Michigan State University, East Lansing, Michigan. "The Chemistry of the

Renewals of Active Grants Approved November 1957 - Cont'd.

- #12R2 - B. L. Freedlander, M.D., Director of Cancer Research, Mount Zion Hospital, San Francisco 15, California. "Experiments on the Carcinogenic and Cocarcinogenic Action of Tobacco Products." \$2,000.00 - Pro rata extension to March 1, 1958.
- #29BR2 - F. Homburger, M.D., President, Bio-Research Institute, Inc., Cambridge 41, Mass. "A Study on the Effects of Various Components of Tobacco and Cigarette Paper Upon the Behavior of Transplantable Tumors." \$25,760.00 - One year
- #50R2 - Pathologic-Anatomic Study of Cellular Changes in Human Lungs. \$1,500.00 - Supplement. For further study of data obtained under the original P.A.S. grant.
- #80R3 - Fellowship Program for 1958 \$36,900.00. This amount includes balance of \$1,900.00 from last year's program. - One year
- #134BR1 - C. M. Pomerat, Ph.D., Professor of Cytology, The University of Texas - Medical Branch, Galveston, Texas. "Tissue Culture Studies of Human Lung." \$12,020.00 - One year
- #137R1 - Samuel Bellet, M.D., Director, Division of Cardiology, Philadelphia General Hospital, Philadelphia 4, Pennsylvania. "Cardiovascular Effects of Smoking and of Nicotine in Experimental Animals and in Human Subjects." \$7,770.00 - One year
- #142R1 - Paul D. Saltman, Ph.D., Assistant Professor, Department of Biochemistry and Nutrition, University of Southern California, Los Angeles 7, California. "Some Aspects of Amino Acid Metabolism in Tobacco Leaves." \$7,560.00 - One year

Renewals of Active Grants Approved February 1958

- #24R2 - H. B. Haag, M.D., Professor of Pharmacology, Medical College of Virginia, Richmond 19, Virginia. "Abstracting and Classifying the Literature on the Biologic Effects of Tobacco." \$7,254.00 - One year
- #50R2 - Pathologic-Anatomic Study of Cellular Changes in Human Lungs. \$750.00 - Addition to supplement approved November 1957. For further study of data obtained under the original P.A.S. grant.

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Renewals of Active Grants Approved February 1958

- #91R3 - Richard J. Bing, M.D., Professor of Medicine, Washington University Medical Service, Veterans Administration Hospital, St. Louis 6, Mo. "The Effect of Smoking on the Coronary Blood Flow and Certain Phases of Myocardial Metabolism in Patients with Arteriosclerotic or Hypertensive Cardiovascular Disease."
\$14,870.00 - One year
- #128R2 - Paul S. Larson, Ph.D., Professor of Pharmacology, Medical College of Virginia, Richmond 19, Virginia. "Enzymatic Transformations of Nicotine."
\$28,287.00 - One year

Renewals of Active Grants Approved May 1958

- #25R3 - Herbert R. Hawthorne, M.D., Chairman, Department of Surgery, Graduate School of Medicine, University of Pennsylvania, Philadelphia 46, Pa. "The Attempted Production of Pulmonary Neoplasms in Experimental Animals by Exposure of the Tracheo-Bronchial System to Tobacco Smoke."
\$4,566.00 - One year
- #117R2 - Victor Richards, M.D., Professor of Surgery, Stanford University School of Medicine, San Francisco 15, California. "A Comparative Study of the Effects of Whole and Fractionated Extracts of Cigarette Smoke and Those of Known Carcinogens on: 1. The Cytology and Nuclear DNA Content of Epidermis in Various Strains of Mice."
\$19,000.00 - One year
- #126R2 - James F. Bonner, Ph.D., Professor of Biology, California Institute of Technology, Pasadena, California. "Enzymatic Study of Methylation Reactions in Plant Tissue."
\$10,005.00 - One year
- #138R1 - Morton I. Grossman, Ph.D., M.D., Associate Clinical Professor of Medicine, University of California Medical Center, Los Angeles 24, California. "The Effect of Smoking on Certain Gastric Functions."
\$8,400.00 - One year
- #141R1 - Alvin I. Kosak, Ph.D., Associate Professor of Chemistry, New York University, Washington Square College, New York, N.Y. "The Isolation and Identification of Certain Lower-Boiling Components of Cigarette Smoke."
\$6,038.00 - One year
- #150R1 - John W. Eckstein, M.D., Assistant Professor, Department of Internal Medicine, College of Medicine, State University of Iowa, Iowa City, Iowa. "Responses of the Peripheral Veins in Man to the Intravenous Administration of Nicotine."
\$2,430.00 - One year.

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Renewals of Active Grants Approved May 1958 - Cont'd.

- #152R1 - Charles B. McCants, Ph.D., Department of Soils, School of Agriculture, North Carolina State College, Raleigh, North Carolina. "Arsenic Content of Soils and Absorption by the Tobacco Plant."
\$3,000.00 - One year
- #155R1 - Duane G. Wenzel, Ph.D., Professor of Pharmacology, The School of Pharmacy, University of Kansas, Lawrence, Kansas. "The Determination of the Chronic Effects of Orally Administered Nicotine on Serum Cholesterol and Phospholipids; the Electrocardiographic Response to Ergonovine; and the Vascular Pathology of Cholesterol-Fed Rabbits."
\$7,705.00 - One year
- #157R1 - R. H. Rigdon, M.D., Professor of Pathology, Director, Laboratory of Experimental Pathology, University of Texas - Medical Branch, Galveston, Texas. "Effect of Tobacco Tar on Respiratory Tract of the Duck."
\$5,278.50 - One year
- #158R1 - Tom G. Bowery, Ph.D., Pesticide Residue Laboratory, Chemistry Department, North Carolina State College, Raleigh, North Carolina. "TDE and Endrin Residues in Cigarette Smoke."
\$4,000.00 - One year
- #159R1 - Cecilie Leuchtenberger, Ph.D., Associate Professor of Cytology and Biology, Institute of Pathology, Western Reserve University, Cleveland 6, Ohio. "A Correlated Histological, Cytological and Cytochemical Study of the Tracheo-Bronchial Tree from Mice Exposed to Cigarette Smoke."
\$21,703.12 - One year

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Renewals of Active Grants Approved November 1957 - Cont'd.

- #12R2 - B. L. Freedlander, M.D., Director of Cancer Research, Mount Zion Hospital, San Francisco 15, California. "Experiments on the Carcinogenic and Cocarcinogenic Action of Tobacco Products." \$2,000.00 - Pro rata extension to March 1, 1958.
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- #50R2 - Pathologic-Anatomic Study of Cellular Changes in Human Lungs. \$1,500.00 - Supplement. For further study of data obtained under the original P.A.S. grant.
- #80R3 - Fellowship Program for 1958 \$36,900.00. This amount includes balance of \$1,900.00 from last year's program. - One year
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Renewals of Active Grants Approved February 1958

- #24R2 - H. B. Haag, M.D., Professor of Pharmacology, Medical College of Virginia, Richmond 19, Virginia. "Abstracting and Classifying the Literature on the Biologic Effects of Tobacco." \$7,254.00 - One year
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Renewals of Active Grants Approved February 1958

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\$14,870.00 - One year
- #128R2 - Paul S. Larson, Ph.D., Professor of Pharmacology, Medical College of Virginia, Richmond 19, Virginia. "Enzymatic Transformations of Nicotine."
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- #25R3 - Herbert R. Hawthorne, M.D., Chairman, Department of Surgery, Graduate School of Medicine, University of Pennsylvania, Philadelphia 46, Pa. "The Attempted Production of Pulmonary Neoplasms in Experimental Animals by Exposure of the Tracheo-Bronchial System to Tobacco Smoke."
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- #150R1 - John W. Eckstein, M.D., Assistant Professor, Department of Internal Medicine, College of Medicine, State University of Iowa, Iowa City, Iowa. "Responses of the Peripheral Veins in Man to the Intravenous Administration of Nicotine."
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Renewals of Active Grants Approved May 1958 - Cont'd.

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- #159R1 - Cecilie Leuchtenberger, Ph.D., Associate Professor of Cytology and Biology, Institute of Pathology, Western Reserve University, Cleveland 6, Ohio. "A Correlated Histological, Cytological and Cytochemical Study of the Tracheo-Bronchial Tree from Mice Exposed to Cigarette Smoke."
\$21,703.12 - One year

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Grants Approved November 1957 - Cont'd.

- #170 - Edward W. Pelikan, M.D., Associate Professor, Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston 18, Mass. "A Study of Structure-Activity Relationships Among Drugs Which Affect Nicotine-Sensitive Physiological Mechanisms."
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\$7,200.00 - One year

Grants Approved February 1958

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\$16,125.00 - One year (This amount includes \$2,000.00 pro rata extension approved November 1957. See grant #12R2.)
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\$7,475.00 - Fifty weeks

Grants Approved May 1958

- #197 - Richard U. Byerrum, Ph.D., Professor of Chemistry, Michigan State University, East Lansing, Michigan. "Biosynthesis of the Pyridine Ring of Nicotine."
\$6,984.00 - One year

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Grants Approved May 1958 - Cont'd.

- #198 - Maurice S. Segal, M.D., Clinical Professor of Medicine, Tufts University School of Medicine, Boston, Mass. "Relationship of Cigarette Smoking to Chronic (Obstructive) Pulmonary Emphysema."
\$5,000.00 - One year
- #203 - Caroline Bedell Thomas, M.D., Associate Professor of Medicine, The Johns Hopkins School of Medicine, Baltimore 5, Maryland.
"a. Studies of Genetic Differences Between Smokers and Non-smokers. b. Studies of Psychological Differences Between Smokers and Nonsmokers As Shown By Comparison of Figure Drawings."
\$11,500.00 - One year

1003537532

TOBACCO INDUSTRY RESEARCH COMMITTEE

August 29, 1958

1958 FELLOWSHIPS

71	Fellowships offered in amount of.....	\$36,300.
	69 Accepted	
	2 Did not answer (Univ. of Kansas & Jefferson)	
65	Paid in amount of.....	33,300.
	(8 for \$600.	
	57 for \$500.)	
	Bal.	<u>\$ 3,000.</u>

Six remain to be paid:

State U. of Iowa Coll. of Med.	\$ 500.
U. of Kansas School of Med.	500.
NYU College of Med.	500.
Western Reserve U. School of Med.	500.
The Jefferson Med. College	500.
Vanderbilt U. School of Med.	500.
	<u>\$3,000.</u>

Thus far, two reports received:

Univ. of Chicago School of Med.	-	6/12
State Univ. of S.D. School of Med.	-	8/22

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Renewals of Active Grants Approved May 1958 - Cont'd.

- #152R1 - Charles B. McCants, Ph.D., Department of Soils, School of Agriculture, North Carolina State College, Raleigh, North Carolina. "Arsenic Content of Soils and Absorption by the Tobacco Plant."
\$3,000.00 - One year
- #155R1 - Duane G. Wenzel, Ph.D., Professor of Pharmacology, The School of Pharmacy, University of Kansas, Lawrence, Kansas. "The Determination of the Chronic Effects of Orally Administered Nicotine on Serum Cholesterol and Phospholipids; The Electrocardiographic Response to Ergonovine; and the Vascular Pathology of Cholesterol-Fed Rabbits."
\$7,705.00 - One year
- #157R1 - R.H. Rigdon, M.D., Professor of Pathology; Director, Laboratory of Experimental Pathology, University of Texas - Medical Branch, Galveston, Texas. "Effect of Tobacco Tar on Respiratory Tract of the Duck."
\$5,278.50 - One year
- #158R1 - Tom G. Bowery, Ph.D., Pesticide Residue Laboratory, Chemistry Department, North Carolina State College, Raleigh, North Carolina. "TDE and Endrin Residues in Cigarette Smoke."
\$4,000.00 - One year
- #159R1 - Cecilie Leuchtenberger, Ph.D., Associate Professor of Cytology and Biology, Institute of Pathology, Western Reserve University, Cleveland 6, Ohio. "A Correlated Histological, Cytological and Cytochemical Study of the Tracheo-Bronchial Tree from Mice Exposed to Cigarette Smoke."
\$21,703.12 - One year

Renewals of Active Grants September 1958

- # 51R3 - H. R. Pratt-Thomas, M.D., Professor of Pathology, Medical College of South Carolina, Charleston 16, South Carolina. "Application of a New Bio-Assay Technique in Examination of Cigarette Smoke Condensate for Possible Carcinogens."
\$6,875.00 - One year
- #134AR2 - Tissue Culture Fellowship - George O. Gey, M.D., Director, Finney-Howell Cancer Research Laboratory, Department of Surgery, The Johns Hopkins Hospital, Baltimore 5, Maryland. "For Studying the Culture of Human Lung Tissue and the Effects of Known and Possible Carcinogenic Agents upon such Tissue."
\$14,835.00 - One year

Renewals of Active Grants December 1958

- #4AR3 - Simon H. Wender, Ph.D., Research Professor of Chemistry, University of Oklahoma - Research Institute, Norman, Oklahoma. "Qualitative and Quantitative Studies of the Individual Polyphenol Content of Cigarette Tobacco and of the Smoke and 'Tars' Resulting from Cigarette Smoking; and Also to Study the Fate of These Compounds in the Animal Respiratory System."
\$15,400.00 - One year

1003537534

Renewals of Active Grants December 1958 - Cont'd.

- # 92R3 - Janet Travell, M.D., Associate Professor, Clinical Pharmacology, Cornell University Medical College, New York 21, New York. "Cardiac Effects of Nicotine in the Rabbit with Experimental Coronary Atherosclerosis."
\$3,437.50 - One year
- #134BR2 - Tissue Culture Fellowship - C. M. Pomerat, Ph.D., Professor of Cytology, The University of Texas - Medical Branch, Galveston, Texas. "For Studying the Culture of Human Lung Tissue and the Effects of Known and Possible Carcinogenic Agents upon such Tissue."
\$12,000.00 - One year
- #134CR1 - Tissue Culture Fellowship - Donald M. Pace, Ph.D., Professor of Physiology and Director, Institute for Cellular Research, University of Nebraska, Lincoln, Nebraska. "For Studying the Effects of Some of the Constituents of Tobacco Smoke on Various Strains of Tissue Cells Cultivated In Vitro."
\$9,750.00 - One year
- #17OR1 - Edward W. Pelikan, M.D., Associate Professor, Boston University School of Medicine, Boston 18, Massachusetts. "A Study of Structure-Activity Relationships Among Drugs Which Affect Nicotine-Sensitive Physiological Mechanisms."
\$10,465.00 - One year
- #174R1 - Russell L. Holman, M.D., Professor and Head, Department of Pathology, Louisiana State University School of Medicine, New Orleans 12, Louisiana. "The Influence of Tobacco Smoking on Acute Myocardial Infarction."
\$7,705.00 - One year
- #18OR1 - B. L. Freedlander, M.D., Director of Cancer Research, Mount Zion Hospital, San Francisco 15, California. "Experiments on the Carcinogenic and Co-Carcinogenic Action of Tobacco Products."
\$16,125.00 - One year
- #181R1 - Benjamin A. Rubin, Ph.D., Baylor University College of Medicine, Houston, Texas. "An Evaluation of the Phenomenon of Tumor Growth Enhancement as an Assay for Carcinogens Among the Polycyclic Hydrocarbons and Related Compounds."
\$9,200.00 - One year

1003537535

Grants Approved May 1958 - Cont'd.

- #198 - Maurice S. Segal, M.D., Clinical Professor of Medicine, Tufts University School of Medicine, Boston, Mass. "Relationship of Cigarette Smoking to Chronic (Obstructive) Pulmonary Emphysema."
\$5,000.00 - One year
- #203 - Caroline Bedell Thomas, M.D., Associate Professor of Medicine, The Johns Hopkins School of Medicine, Baltimore 5, Maryland. "a. Studies of Genetic Differences Between Smokers and Nonsmokers. b. Studies of Psychological Differences Between Smokers and Nonsmokers as Shown by Comparison of Figure Drawings."
\$11,500.00 - One year

Grants Approved September 13-14, 1958

- #194 - Donald M. Pace, Ph.D., Professor and Chairman, Department of Physiology and Director, Institute for Cellular Research, University of Nebraska, Lincoln, Nebraska. "To Study the Effects of Certain Constituents of Tobacco Smoke on Tissue Cells Cultivated in Vitro."
\$8,000.00 - One year
- #208 - Pauline Heizer, Ph.D., Research Associate in Cytology and Cytochemistry, Stanford University School of Medicine, San Francisco 15, California. "A Comparative Study of the Early Histological and DNA Changes in the Epidermis of Two Strains of Mice (C57 Blacks and Swiss Websters) After Daily Applications of Whole Cigarette Smoke Condensate (Alone and Combined with Croton Oil) and the Carcinogens 20-Methylcholanthrene and 3:4 Benzpyrene."
\$22,000.00 - One year. Terminal.
- #209 - Sydney C. Rittenberg, Ph.D., Professor of Bacteriology, University of Southern California, Los Angeles 7, California. "The Bacterial Degradation of Nicotine and Related Compounds. The Objective of the Project is the Elucidation of the Intermediary Metabolism of Nicotine Oxidation."
\$10,152.00 - One year
- #212 - F. Homburger, M.D., President and Director, Bio-Research Institute, Inc. Cambridge 41, Massachusetts. "Further Studies on Carcinogens."
\$28,760.00 - One year

Grants Approved December 8-9, 1958

- #200 - Philip Cooper, M.D., Chief, Surgical Service; Clinical Professor of Surgery, Veterans Administration Hospital, Bronx 68, New York. "A Study of the Effect of Extracts of Tobacco on Cultures of Tumor and Normal Cells. Animal Transplants of Tumor Tissue from Tissue Cultures."
\$17,500.00 - One year

1003537536

Grants Approved December 8-9, 1958 - Cont'd.

- #206 - Edward F. Domino, M.D., Assistant Professor of Pharmacology, University of Michigan, Ann Arbor, Michigan. "Effects of Tobacco Smoke and Nicotine on the Central Nervous System."
\$11,500.00 - One year
- #213 - Marion B. Sulzberger, M.D., Professor and Chairman, Department of Dermatology and Syphilology, New York University - Bellevue Medical Center, New York 16, N.Y. "Investigation of the Effects of Tobacco on the Human Vascular System, Based on the Fact that Certain Tobacco Effects are Due to Allergic Susceptibility of Specific Individuals Rather than to Obligatorily Toxic Products in Tobacco Smoke. And that Patients with Occlusive Vascular Diseases Respond Differently than Healthy Smokers."
\$15,000.00 - One year
- #214 - Carl C. Seltzer, Ph.D., Research Fellow in Physical Anthropology, Harvard University, Cambridge, Massachusetts. "Morphology and Smoking in College Graduates: A Fifteen-Year Follow-Up Study."
\$4,955.00 - One year

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